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TITLE: Manganese Research Health Project (MHRP)

PRINCIPAL INVESTIGATOR: Michael Aschner, Ph.D.

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, TN 37203

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14. **ABSTRACT**
The Administrative Core includes Dr. Michael Aschner of Vanderbilt University Medical Center as the Principal Investigator (PI), and an Administrative Assistant. The Administrative Core is involved in all facets of the MHRP, ensuring proper allocation of funds to the various Research and Support Cores, required financial reporting to the Army, facilitation of the communication between the various CPIs (Core Principal Investigators) , timely reporting of the results, communication with all relevant Department of Defense (DoD) personnel, resolving issues related to conflict of interest, and general coordination between the various Cores. A key early objective of the Administrative Core is to allocate, at the direction of the Steering Committee, remaining year 1 funds to one or more additional research cores identified. The Administrative Core is advised by the CPIs of the MHRP. Advice includes issues of scientific merit, oversight of ethical issues associated with animal and human subjects, review of potential additional projects, as well as review of funded projects and progress reports.

15. SUBJECT TERMS
Manganese, neurotoxicology, iron deficiency, welding, manganese mining, nutrition

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Table of Contents

Introduction and Body

The Steering Committee of the MHRP met on March 30, 2005 to review the proposals submitted for the Phase II of the project. The Steering Committee solicited pre-proposals in the fall of 2004. We received a total of 28 pre-proposals (see pp 5-6), and the scientific merit of each of those was discussed at a Steering Committee meeting that was held at Vanderbilt University in Nashville on December 7, 2004.

Of the 28 pre-proposals eight were selected for further consideration (see page 7), and each of the principal investigators of these pre-proposal was requested to submit a full proposal following the NIH submission guidelines. The proposals were due on March 1, 2005, and on March 30, the Steering Committee reviewed each of these applications at a special meeting in Washington, DC. Prior to the meeting, I assigned at a minimum a primary and secondary reviewer for each application, and each of the reviewers was requested to provide a full written critique. During the meeting we followed a routine study section approach, in which each proposal was reviewed by the primary reviewer, and additional comments were contributed by the secondary reviewer (or tertiary, where appropriate), followed up by an open discussion of the full committee.

The MHRP Steering Committee is chaired by Ms. Anne Tremblay, the Secretary General of the International Manganese Institute (IMnI), and its members include Dr. Barbara Beck of Gradient Corporation, Dr. Tomas Guilarte of the Johns Hopkins University, Dr. Steven Seilkop of SKS Consulting, Dr. Joan Cranmer of University of Arkansas, Dr. Leonard Levy, MRC Institute for Environment and Health University of Leicester, UK, Dr. Jerry Roper of Afton Chemicals, Mr. Christian Plazanet of Eramet, Mr. Jeff Leader of BHP Billiton, and Mr. John Hilbert of Kinghorn, Hilbert & Associates. The MHRP Organizational Chart is shown on page 8.

In addition, we have hired an Administrative Assistant; Ms. Alycia Buford-Penn. Ms. Buford-Penn joined the MHRP in May 2005. She has trained at Vanderbilt to become versed in all facets of administrative issues germane to this program. She has facilitated communications between the various CPIs and the PI, and has attended to all the administrative issues associated with the MHRP, including reimbursements, reservations for trips, dissemination of pertinent information, etc.

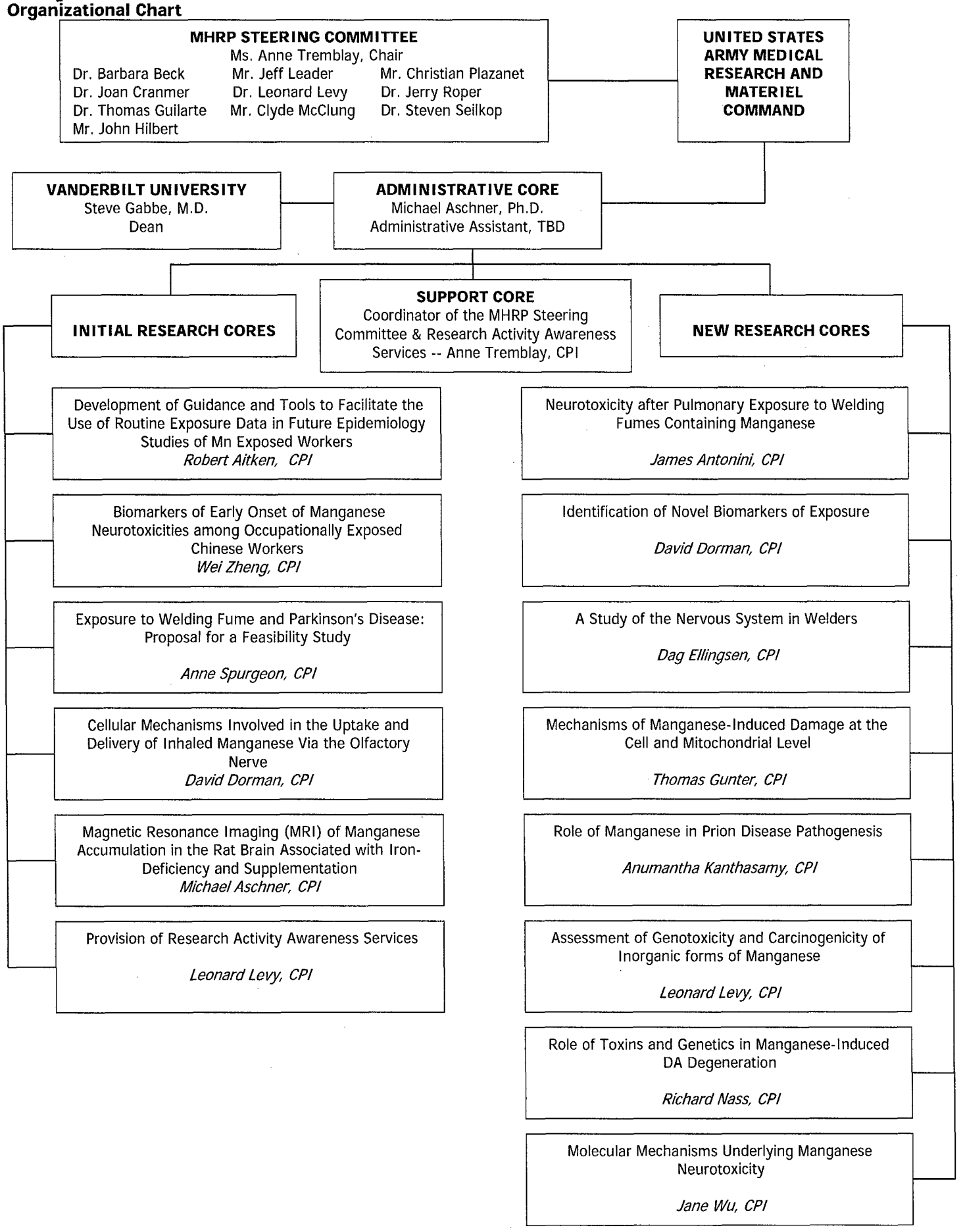
SUBMITTER	INSTITUTION	PROPOSAL TITLE	ESTIMATED COST	ESTIMATED TIMELINE
Philip Holmes	Sr. Toxicologist & Project Resource Mgr / MRC Institute for Environment & Health	Assessment of the Published Database on Genotoxicity and Carcinogenicity of Inorganic Forms of Manganese, and Implications with regard to OECD Regulatory Guidance on the Assessment of the Genotoxic Potential	$20,100	1 Year
Jane Wu	Professor, Dept of Pediatrics, and Cell and Developmental Biology, and Pharmacology / Vanderbilt University Medical Center	Developing Biomarkers for Manganese Toxicity	$300,000	2 Years
Thomas Gunter	Professor of Biochemistry & Biophysics / University of Rochester Medical Center	Mechanisms of Manganese-Induced Damage at the Cell and Mitochondrial Level	$200,000	3 Years
David C. Dorman	Director, Division of Biological Sciences / CIIT Centers for Health Research	Identification of Novel Biomarkers of Manganese Exposure	$285,033	1 Year
James Antonini	Health Effects Laboratory Divison / National Institute for Occupational Health & Safety	Welding fume generation / Long-term toxicological studies	$386,950	3 Years
Anumantha Kanthasamy	Parkinson's Disorder Research Laboratory / Iowa State University	The Role of Manganese in Prion Disease Pathogenesis	$179,928	3 Years
Richard Nass	Center for Molecular Neuroscience / Vanderbilt University Medical Center	Role of Toxins and Genetics in Manganese-Induced DA Neuron Degeneration	$160,000	2 Years
Dag Ellingsen	Senior Physician / National Institute of Occupational Health, Norway	Study on Manganese-related Health Effects in Welders and in Patients with Manganism	$201,300	4 Years
Brian Gulson	Graduate School of the Environment / Macquarie University	Exposure and Other Assessments of Occupationally Exposed Groups	$338,785	1 Year
Brian Gulson	Graduate School of the Environment / Macquarie University	Exposure and Other Assessments of Environmentally Exposed Groups	$234,316	2 Years
Jerome A. Roth	Department of Pharmacology and Toxicology / University of Buffalo	Research on Toxic Manifestations of Manganese	$555,000	3 Years
Jill K. Hiney	Department of Veterinary Integrative Biosciences / Texas A&M University	Effects of Low Dose Manganese Exposure on Male Puberty	$300,000	3 Years

Anne Spurgeon	Institute of Occupational and Environmental Medicine / University of Birmingham	The Investigation of the Possible Association between Exposure to Welding Fume and Parkinson's Disease: Outline Proposal for a Case-control Study	$765,594	3 Years
Rosemarie Bowler	SFSU	An Evaluation of Neurological and Neuropsychological Status of Active Welders to Determine Early Health Effects of Manganese Exposure in Welders	$300,000	2 Years
Mary Beth Genter	Department of Environmental Health and Center for Environmental Genetics / University of Cincinnati	Manganese Transporters as a Basis for Accumulation of Mn and Mn-induced Toxicity in the Central Nervous System	$255,300	2 Years
Bradley Klein	Dept. of Biomedical Sciences & Pathobiology / Virginia Tech	Relative Modulation of the Motor and Cognitive Sequelae of Parkinsonism by Manganese	$82,326	2 Years
Richard Mailman	Neurosciences Hospital of North Carolina School of Medicine / Chapel Hill	Determination of whether D1 Agonists are as Effective in Manganese Toxicity as in MPTP-parkinsonism, Parkinson's Disease, or Cognitive Dysfunction	$366,250	2 Years
Ken Hudnell	US EPA	Effect of Airborne Manganese Exposure on Elementary School Children	$200,000	2 Years
John Ross	Department of Environmental and Occupational Medicine / University of Aberdeen	Determination of the neurological substrate of mild cognitive impairment in underwater divers who have worked as welders.	$293,100	2 Years
Catherine J. Price	RTI International	Neurobehavioral Methods and Models of Manganese Toxicity	$50,000	1 Year
Martin Glen	Jerry Pettis Memorial Veterans Medical Center	Effects of Manganese on Hearing	$615,914	3 Years
Timothy Bates	School of Biomedical Sciences / University of Nottingham	An Investigation of the Molecular Mechanisms of Manganese-Induced Mitochondrial Dysfunction in the Brain	$470,296	3 Years
Julia D. George	Life Sciences & Toxicology / RTI International	Effects of Manganese on Male Reproduction	$70,000	6 Months
Nikolay Filipov	College of Veterinary Medicine / Mississippi State University	Role of Chronic Lung Inflammation and Age in the Neurotoxic Effects of Long-term, Low-level Exposure to Manganese	$145,860	2 Years
Janis O'Donnell	University of Alabama	Effect of Genetic Variation in Dopamine Regulating Genes in Drosophila on Susceptibility to Manganese-induced Biological Damage	$148,950	2 Years
Cai Jiyang	Vanderbilt Eye Institute / Vanderbilt University	Biomarkers of Oxidative Stress Associated with Chronic Manganese Toxicity	$65,075	1 Year
Dan Nebert	University of Cincinnati Medical Center	Genetics of Manganese Reproductive Toxicity	$170,231	1 Year
Marianne Hopkins	Maine Medical Center	Manganese Exposure, Genetic Factors, and CNS Deficits in Welders	$391,346	2 Years
TOTAL FOR ALL PROJECTS			**$7,551,654**	

Eight Finalist Projects Recommended for Funding

1. **Neurotoxicity after Pulmonary Exposure to Welding Fumes Containing Manganese - Antonini, James, PI**
2. **Identification of Novel Biomarkers of Manganese Exposure - Dorman, David, PI**
3. **A Study of the Nervous System in Welders - Ellingsen, Dag, PI**
4. **Mechanisms of Manganese-Induced Damage at the Cell and Mitochondrial Level - Gunter, Thomas, PI**
5. **Role of Manganese in Prion Disease Pathogenesis - Kanthasamy, Anumantha, PI**
6. **Assessment of Genotoxicity and Carcinogenicity of Inorganic-forms of Manganese - PI Levy, Leonard, PI**
7. **Role of Toxins and Genetics in Manganese-Induced DA Degeneration - Nass, Richard, PI**
8. **Molecular Mechanisms Underlying Mn Neurotoxicity - Wu, Jane, PI**

Organizational Chart



MHRP Symposium at the XXII International Neurotoxicology Conference on Environment and Neurodevelopmental Disorders.

The MHRP also supported a full-day symposium to take place at the XXII International Neurotoxicology Conference on Environment and Neurodevelopmental Disorders. The Conference took place September 11-14, 2005 at Research Triangle Park, NC. A copy of the program is enclosed in the page 10. Dr. Joan Cranmer, a member of the MHRP Steering Committee and the Editor of Neurotoxicology has kindly agreed to allocate a full-day for 2 sessions that addressed contemporary issues of Mn. The goal of this symposium was to discuss recent advances in our understanding of molecular mechanisms governing the transport of Mn in the brain, the effects of Mn on specific neuronal systems, the resulting behavioral effects in non-human primates and the discovery of novel biomarkers of Mn exposure in humans. The speakers described a broad spectrum of model systems from the worm *C. elegans*, to mice, non-human primates and humans in a comprehensive approach to increase our understanding of determinants of Mn neurotoxicity. This symposium was multidisciplinary in nature bringing together scientists with expertise in behavioral, molecular, brain imaging and human population studies in the context of mechanistic investigations of Mn neurotoxicity. Participation in this symposium enabled the audience to become acquainted with the latest information and scientific breakthroughs in this fast-paced research area and provided information germane to risk analysis.

In addition, a meeting of all MHRP researchers (CPIs and several members of the Steering Committee took place after the scientific sessions on Wednesday evening (September 11, 2005). The purpose of this meeting was for brainstorming on Mn related issues with an attempt to streamline projects, develop new venues for scientific studies, and assess the possibility of forming collaborative studies between the groups. The meeting was highly successful. The minutes of the meeting are enclosed on page 12.

Reportable Outcomes

- Phase I of the MHRP commenced in February 2005
- Additional projects were identified, and a total of 8 new projects will commence at the latest February 2006.
- The Steering Committee met on several occasions to discuss the progress of the MHRP.
- A meeting was organized for MHRP investigators in which there projects were discussed and initial data presented (where applicable).
- An administrative Assistant was hired.

Conclusions

The administrative Core of the MHRP is fully functional. It is successfully involved in all facets of the MHRP, ensuring proper allocation of funds to the various Research and Support Cores, facilitation of the communication between the various CPIs, timely reporting of the results, communication with all relevant Department of Defense (DoD) personnel, resolving issues related to conflict of interest, and general coordination between the various Cores. The Steering Committee has provided input to the MHRP identifying project worthy of funding, and these should commence within the next few weeks.

References

Not applicable

PROGRAM

Wednesday Morning 14 Sept 2005 8:30 AM – 12:00 NOON

Symposium

SESSION IX-A: CONTEMPORARY HEALTH ISSUES ASSOCIATED WITH OVER EXPOSURE TO MANGANESE

Session Chairs: Michael Aschner and Thomas Gunter

Theme: This multidisciplinary session will address contemporary research issues associated with the health effects of manganese (Mn) both in humans and animal models. Speakers will discuss recent findings on the specific cellular, molecular, and physiologic mechanisms by which manganese mediates its adverse effects. Speakers will also note factors, such as age, pre-existing disease, and genetics, as conditions that might predispose individuals to enhanced susceptibility to manganese toxicity. The session will span studies in various tissue culture models to non-human primates, incorporating diversity of techniques, from molecular biology to imaging.

Timely Topics to be Addressed:

- Consideration of the relevant heath issues associated with over exposure to manganese.
- Characterization of exposures
- Development of appropriate biomarkers of exposure.
- Quantifying the relationships between exposure and ill health, including pharmacokinetics.
- Understanding the mechanisms of transport, damage and repair.
- Understanding and utilizing invertebrate models such as the c. elegans to probe for mechanisms of Mn neurotoxicity

8:30 – 8:40 AM
Introduction
Co-Chairs: Michael Aschner and Thomas Gunter

8:40 – 9:10 AM
Factors that Influence the Pharmacokinetics of Inhaled Manganese
David Dorman, *CIIT, Research Triangle Park, NC*

This presentation will discuss manganese inhalation exposure conditions that result in manganese accumulation within the brain of adult nonhuman primates as well as in fetal, juvenile, adult, and aged rodents. Dr. Dorman will discuss the pharmacokinetics of inhaled manganese and the effect of particle solubility on this process.

9:10 – 9:40 AM
Dietary Iron Modulates Manganese Neurotoxicity
Michael Aschner, *Vanderbilt University*, Nashville, TN

Manganese and iron are essential metals for normal growth and development. Both metals compete for and share the same cellular transporters. Thus, during periods of low dietary Fe intake, the transport and deposition of Mn in the brain are increased. This presentation will address magnetic resonance (MR) studies monitoring the accumulation of brain Mn when dietary Fe levels are modulated.

9:40 – 10:10 AM
Characterization of Welding Fumes and their Neurotoxic Effects
James Antonini, *NIOSH, Morgantown, WV*

Dr. Antonini will report on recent characterization of welding fume metal composition and particle size. He will also address the pulmonary and neurotoxic effects of animals exposed to welding fumes by inhalation.

10:10 – 10:30 AM Break

10:30 – 11:00 AM
Discovery of Biomarkers of Manganese Exposure in Humans
Wei Zheng, *Purdue University, IN*

This presentation will discuss the possibility of using blood levels of manganese, iron, or iron metabolism-associated proteins as biomarkers for manganese toxicity based on human studies of welders with occupational exposure to manganese in welding fume. The outcomes of clinical intervention with chelating agents will also be discussed.

11:30 AM – 12:00 NOON
Neurochemical Changes in the Living Non-human Primate Brain following Chronic Mn Exposure
Tomas Guilarte, *Johns Hopkins University*

This presentation will deal with the effects of low level chronic manganese exposure on behavioral, imaging and neuropathological endpoints in the non-human primate brain. The speaker will detail early onset neurobehavioral changes in monkeys exposed to manganese, highlighting changes in multiple neurotransmitter systems and motor function related to alterations in the dopaminergic, glutamatergic, and GABAergic systems.

11:30 AM – 1:30 PM Lunch Break

Wednesday Afternoon - 14 Sept 2005 1:30 PM – 4:00 PM

Symposium - continued

SESSION IX-A: CONTEMPORARY HEALTH ISSUES ASSOCIATED WITH OVER EXPOSURE TO MANGANESE

Session Chairs: Tomas Guilarte and Anumantha Kanthasamy

1:30 – 2:00 PM

Mitochondrial Effects of Manganese

Thomas Gunter, *University of Rochester, Rochester, NY*

Mn^{2+} is known to be readily sequestered by mitochondria including neuronal cells. Furthermore, Mn^{2+} is also known to bind to almost every Ca^{2+} binding site, usually more strongly than Ca^{2+} itself. Dr. Gunter will discuss mechanisms by which Mn^{2+} may cause cell damage by interfering with Ca^{2+} activation of ATP production and inhibition of mitochondrial enzymes.

2:00 – 2:30 PM

The Role of Prion Protein in Manganese Neurotoxicity

Anumantha Kanthasamy, *Iowa State University*

Altered Mn is known sequelae of prion disease, but little is known about the role of Mn in this disease. Dr. Kanthasamy will address the binding of Mn to cellular prion protein and the potential role it plays in the pathogenesis of sporadic prion disease.

2:30 – 3:00 PM **Break**

3:00 – 3:30 PM

Manganese-induced Dopaminergic Neuron Degeneration in *C. elegans*: A Pharmacogenetic Analysis

Richard Nass, *Vanderbilt University, Nashville, TN*

Manganese (Mn^{2+}) neurotoxicity resembles a number of aspects of the dopamine (DA) neuron degenerating disorder Parkinson's disease (PD). Expression of the pre-synaptic protein α-synuclein and the oxidative stress-induced protein parkin has been proposed to contribute to the pathogenesis of both disorders. Dr. Nass will discuss a novel pharmacogenetic model using the genetically tractable nematode *C. elegans* to dissect and characterize the molecular components involved in DA neuron degeneration and its utility in characterizing Mn-induced neurotoxicity

3:30 – 4:00 PM

Title TBA

Dag Ellingsen, National Institute of Occupational Health, Oslo, Norway

A Study of the Nervous System in Welders

Dag Ellingsen,

The speaker will address investigations of neurological effects in manganese-exposed workers in Russia: 1) a cross-sectional study of 96 welders and age-matched referents, and 2) a clinical study of 27 manganism patients. The results of these will detail associations between degradation of neurobehavioral endpoints (*e.g.,* digit span, finger tapping test scores) and level of manganese exposure (as measured in blood and urine) in welders.

MHRP The Manganese Health Research Program	
Date: Thursday, Septembe r 14, 2005	**Location:** Sheraton Imperial Hotel & Conference Center, Triangle Park, North Carolina
Time:	4:00pm – 7:00pm
Attendees: Antonini, James; Aschner, Michael; Dorman, David; Ellingsen, Dag; Guilarte, Tomas; Gunter, Thomas; Hilbert, John; Kanthasamy, Anumantha; Lukey, Brian; Nass, Richard; Tremblay, Anne; Vigneulle, Roy; Zheng, Wei; Roper, Jerry; Fitsanakis, Vanessa, Cranmer, Joan, Buford, Alycia	
Regrets: Leonard Levy	

4:00 – 4:10 .....Introductions

Dr. Aschner opened the meeting with introductions of Guest Panel & MHRP members by introducing themselves

4:10 – 4:20 PM (POWERPOINT PRESENTATION)
MHRP - Origin and Industry Perspective
Anne Tremblay, Secretary General, International Manganese Institute, Paris

Anne Tremblay reviewed the origins of the MHRP, and described industry's expectations for the program.

- International Manganese Institute's Annual Review 2004 was passed out to everyone
- IMnI @ a Glance: Promote & Support the Development & Use of Manganese
- IMnI Priority: To encourage research on how Mn affects human health, in order to better protect industry's workers.
- IMnI founded in 1975, based in Paris, France. Sixty+ members, operating in 29 countries – China represents ¼ of membership.
- IMnI initiated and funded the lobbying effort that made the MHRP possible. Final aim is to raise $6M for a 3-year program.
- MHRP funded through DoD – program has so far received $3.65M over past 2 fiscal years ('04 & '05)

4:20 – 4:35 PM
Lobbying Efforts John Hilbert, President, Kinghorn, Hilbert & Associates, LLC, Washington, DC

Attorney John Hilbert briefed the attendees of the process associated with his firm's lobbying. Key Senators and Congressmen that have supported the effort were identified, and it is hopeful that they will continue to champion the MHRP for the 07 budget.

Update Funding for MHRP, **FY 04, $1.4 million, FY 05, $2.25 million** and the request put forth for **FY 06 is $4.1 million**

4:35 – 4:50 PM. (POWERPOINT PRESENTATION)
Military Relevance of MHRP and Expectations for Deliverables
Col. Brian Lukey, USAMRMC
Ft. Detrick, Maryland

Col. Lukey is the director of MOMRP (**Military Operational Medicine Research Program**) and has been in the program for 2 years. MOMRP mission is to provide biomedical solutions that protect & enhance soldier performance & health. Col. Lukey detailed his responsibilities and alluded to his interest in the MHRP given his previous training and expertise in Toxicology. Col. Lukey discussed issues related to USAMRMAC inner workings, and the requirement that are needed for the release of funds to grantees.

4:50 – 5:10 PM (POWERPOINT PRESENTATION)
MHRP - Scientific Perspective
Michael Aschner, Vanderbilt University Medical Center

Dr. Aschner went over the following objectives of the MHRP proposal.

- define the scope of the contributions of environmental and occupational manganese exposure to health, disease and dysfunction;
- identify and investigate factors, such as age, nutritional deficiencies, pre-existing disease, and genetics, that make individuals more susceptible to the effects of manganese;
- develop common exposure assessment protocols and exposure reconstruction methodologies;
- determine whether manganese plays role in increasing the relative risk for the development of idiopathic Parkinson's disease (IPD) in welders;
- Identify biomarkers for the diagnosis of the potential adverse effects of manganese, taking account of other factors such as diet (i.e. iron deficiency);
- understand the physiological mechanisms that govern manganese accumulation within the brain,, with special emphasis on the role of olfactory transport of the metal;
- provide new modalities for the treatment of excessive manganese exposure (i.e. iron repletion);
- provide data to health forum regulators on which sound regulatory and risk assessment may be based;
- provide timely research activity awareness services to health professionals and the manganese industry and its workers;
- support innovative, multidisciplinary research in humans and animal models, on the specific cellular, molecular, and physiologic mechanisms by which manganese mediate possible adverse health effects.

Dr. Aschner expressed hope that through discussions between the MHRP grantees the total return on the project will exceed the sum of individual projects through potential collaboration. He suggested that the MHRP will assist in educating a new generation of scientists and hopefully also lead to additional funding through federal agencies such as the EPA and NIH. He expressed his enthusiasm for the program and his hope that the MHRP will have a major impact in years to come when issues related to Mn risk assessment are revisited.

5:10 – 6:00 PM - Open Discussion - All participants

- Col. Lukey closing remarks-panel in the Army review proposals instead of independent group, stated to go to website: www.momrp.org

- Having a website link & list projects, send language to Dr. Aschner on what to put on website

- Dr. Aschner ask when will funds be released, will try to expedite it per Dr. Roy Vigneulle

7:00PM - MHRP Dinner Indian Place Restaurant via Hotel Shuttle
919-460-3339

Attendees: Aschner, Michael; Ellingsen, Dag; Gunter, Thomas; Hilbert, John; Kanthasamy, Anumantha; Nass, Richard; Tremblay, Anne; Zheng, Wei; Roper, Jerry; Michael Taylor; Christian Plazanet

CORE 1

Development of Guidance and Tools to Facilitate the Use of Routine Exposure Data in Future Epidemiology Studies of Manganese Exposed Workers

Dr Robert J. Aitken
Institute of Occupational Medicine
Edinburgh, UK EH14 4AP

ABSTRACT:

In the manganese production industry, several companies routinely collect information on exposures in the workplace, and several carry out health surveillance. However, many different approaches are used. These include differences in the use of personal and static monitoring, collection of different size fractions (respirable, inhalable, total), different analysis methods, (gravimetric, ICP, species) and differences in data metrics, storage, traceability and quality assurance procedures. Often, little contextual data is retained. Diverse approaches also characterize the assessment of health end points. These differences limit the utility of such data. In this study we will(i)review methods for the evaluation of neurological health end points and to develop recommendations for a core set of evaluation methods and (ii) identify, develop and evaluate a set of methods, guidelines and tools to enable manganese producer companies to routinely collect valid, appropriate and comparable information relating to manganese exposure, applicable to current and future, as yet unplanned, epidemiological studies.

Introduction

In the manganese production industry, several companies routinely collect information on exposures in the workplace, and several carry out health surveillance. However, many different approaches are used. These include differences in the use of personal and static monitoring, collection of different size fractions (respirable, inhalable, total), different analysis methods, (gravimetric, ICP, species) and differences in data metrics, storage, traceability and quality assurance procedures. Often, little contextual data is retained. Diverse approaches also characterize the assessment of health end points. These differences limit the utility of such data for exposure assessment and exposure reconstruction required for high quality epidemiology, particularly where a multi-centre approach is being developed.

In this project, we propose(i) to review methods for the evaluation of neurological health end points in the manganese industry and in other industries where these end points are considered relevant and to develop recommendations for a core set of evaluation methods to be used for the evaluation of these end points and (ii) to identify, develop and evaluate a set of methods, guidelines and tools to enable manganese producer companies to routinely collect valid, appropriate and comparable information relating to manganese exposure, applicable to current and future, as yet unplanned, epidemiological studies.

The contract for this project from Vanderbilt University states that the agreement shall become effective on 1st May 2005. However formal exchange of contracts did not occur until 14th of September 2005 due to difficulties in establishing whether the project fell within the definition of a human use study and the status of IRB approval.

This report therefore reflects progress since the effect start of the project on 1st October 2005.

Body

As detailed in the proposal, the main tasks and deliverables identified for this work are as follows.

Table 1 Tasks and Deliverables

	Task	Deliverable
1	Review of health endpoints	Report
2	Measurement strategy - development, validation and recommendation	Guideline document
3	Measurement methods - development, validation and recommendation	Guideline document
4	Database - development and validation	Database and guideline document
5	Dissemination	Workshop
6	Development of Proposal for Q/A audit system	Proposal
7	Reporting	

At this stage of the project (3 months) tasks 1- 4 were planned to have started, but no deliverables yet provided. Most progress has been made in relation to task 4. Progress in relation to each of these is now described.

Task 1. Review of health endpoints

Since neurobehavioral performance is one of the outcomes of choice when investigating the early effects of manganese exposure it is important to ensure the selection of the most appropriate (and economical) set of tests.

In this respect a number of aspects of neurobehavioural tests require examination and assessment. The most important of these are the following:

- The degree to which tests are culturally specific. This is likely to be of less concern in respect of motor tests than, for example verbal tests, but nevertheless requires consideration.
- The reliability (in the sense of repeatability) of the tests and the associated importance of establishing methods to define the inter- and intra- reliability of the testers.
- The sensitivity and specificity of the tests in relation to the identification of early signs of the health outcome of interest.

It is proposed therefore to examine each of the above aspects in relation to those tests considered relevant to the field. This will include tests which have been used in existing studies of the health effects of manganese exposure, and other tests identified as incorporating a strong motor component. The work will be carried out by means of an examination of any existing published data relating to the initial development and subsequent use of the selected tests. Important aspects of the data in this respect will include scores obtained in different cultural settings, with different age-groups, different patient groups and from control groups. The objectives would be to identify the most appropriate tests for any future study and to define any limitations of the tests which will require consideration when interpreting the results.

No progress has been made with this task. The consultant identified to carry out this task at the time of the proposal has subsequently retired and is not able to take forward the task. Other possibilities are currently being investigated.

Task 2. Measurement strategy - development, validation and recommendation

In this task we will develop, evaluate and refine generic strategies to demonstrate compliance and to collect sufficient data on which future epidemiology studies could be based. Designs will be based on state the art exposures assessment approaches. Designs will also be flexible so as to facilitate comparisons between size-

dependent measurement fractions (inhalable, thoracic, respirable) or to carry out comparisons between measurement methods. These strategies will be incorporated into a guideline document which will be a deliverable for this task.

Task 3. Measurement methods - development, validation and recommendation

Various sampling methods are currently used in routine programs to assess exposure to manganese (IEH/IOM 2004). These methods include the use of personal samplers such as the Millipore 37mm cassette, the IOM inhalable sampler (Mark and Vincent 1986) and various forms of cyclone. In some cases, only static (fixed) samples are taken.

Based on the outcome of the recent manganese criteria document (IEH/IOM 2004) we consider that it is appropriate that measurement methods are based on personal sampling of inhalable and respirable manganese, supplemented by limited static sampling to determine manganese species. We currently consider that a two-fraction sampler variant of the IOM inhalable sampler (Aitken *et al;*1993) (for respirable and inhalable) may be the most appropriate for personal sampling. The utility of a sampler of this type has been investigated in the nickel industry (Aitken and Hughson 2005) but not yet for manganese. It is planned that the recommended methods will be tested as part of the field study in the proposed epidemiological study of Chinese manganese workers (Zheng, Core2), also funded by the MHRP programme. The field study will comprise evaluation of the utility in the sampler and a subset of the study designs and comparison of the inhalable and respirable fractions. Personal samples collected will be evaluated gravimetrically, to determine total mass and by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) to determine total manganese. Static samples will be analysed to determine manganese species, according to the method described by Ellingsen *et al;* (2003).

However, given recent concerns about the potential impacts of ultrafine particles (or nanoparticles) on health (e.g. Royal Society 2004) and the apparent potential for translocation of these particles along the olfactory nerve into the olfactory bulb (e.g. Oberdorster *et al;*2004) we now consider that it would be important to obtain information on exposure to these particles.

Currently it is recognised that measurement of this fraction of aerosol is very challenging (Aitken *et al;* 2004) and that there are no agreed methods. Table 2 summarises the main methods an approaches and some of the difficulties.

Table 2 Summary of methods

Metric measured	Method	Comment
Mass	Size selective personal sampler	No current devices with a size fraction cut-off in the nm size range but could be developed Static device would overcome limit of detection issues
Number	Optical Particle Counter	Particles smaller than 300 nm not detected
	Condensation particle counter (CPC)	Real time number concentration, up to 1000 nm
	Scanning mobility particle sizer (SMPS)	Real-time Real-time size selective, based on mobility diameter 3 – 800 nm
	Electrical low pressure impactor (ELPI)	Real time size selective (aerodynamic diameter) detection of number concentration. Sample collection

Surface area	Epiphaniometer	Radioactive tagging based on (Fuchs) surface area
	BET bulk analysis	Estimates based on gas adsorption
Image analysis	SEM/TEM	Analysis of morphology Samples may be collected by personal samplers or size selective static samplers

As part of our field exercise we will evaluate the usefulness of particle counting methods (CPC) in assessing exposure to ultrafine particles in manganese production scenarios.

Task 4. Database - development and validation

Use of a well developed exposure database plays a significant role in maximising the value of exposure data. A database, along with relevant documentation can be used to enable consistency of data, in terms of the type of information collected, the way in which it is collected, and the creation of appropriate data summaries and reports. When the same databases are used in different plants, accessibility and comparability of data is greatly enhanced. An important aspect is the collection of contextual data including environmental conditions and working practices.

Substantive progress has been made towards development of and interactive database which will be used to hold collected information. The database is based on the development of a generic database in development for the European Chemical industry (Ritchie *et al;* 2003).

Figure 1 provides a very generalized view of the key data elements that will be collected in the database, and how different areas of data are linked together. For each area and data element, a much more detailed data specification is produced and implemented as tables that will hold actual data in the database. Examples of more detailed entity-relationship diagrams for Company/Premises level and Exposure survey level are shown in Figures 2 and 3, respectively.

A key point to note is that many of the data elements and their data can be shared between different areas of the database, and not repeatedly entered. For example, Workplace, Process and Task information that has been entered once at Premises level can later be repeatedly referenced, say when entering data about the scenario of worker exposure during an exposure survey. Similarly, data about products and hazardous substances (ingredients) can be built up to be used by Premises, Surveys or other risk assessments alike.

Besides reducing data-entry this allows the bank of related data to be built up and so can facilitate more detailed and sophisticated analysis to be performed. For example, after suitable data is entered, a report could be specified that analysed or grouped exposure results by hazardous compound X; or by workplace Y; or Process Z; or some other combination of these, and so on.

To date we have implemented prototype tables at the Company, Premises and Exposure Survey levels. For a flavour of this work to date Figures 4 and 5 show prototype screenshots of interest.

Manganese Exposure DB: Overall Schematic of Proposed Key Data Elements In Database

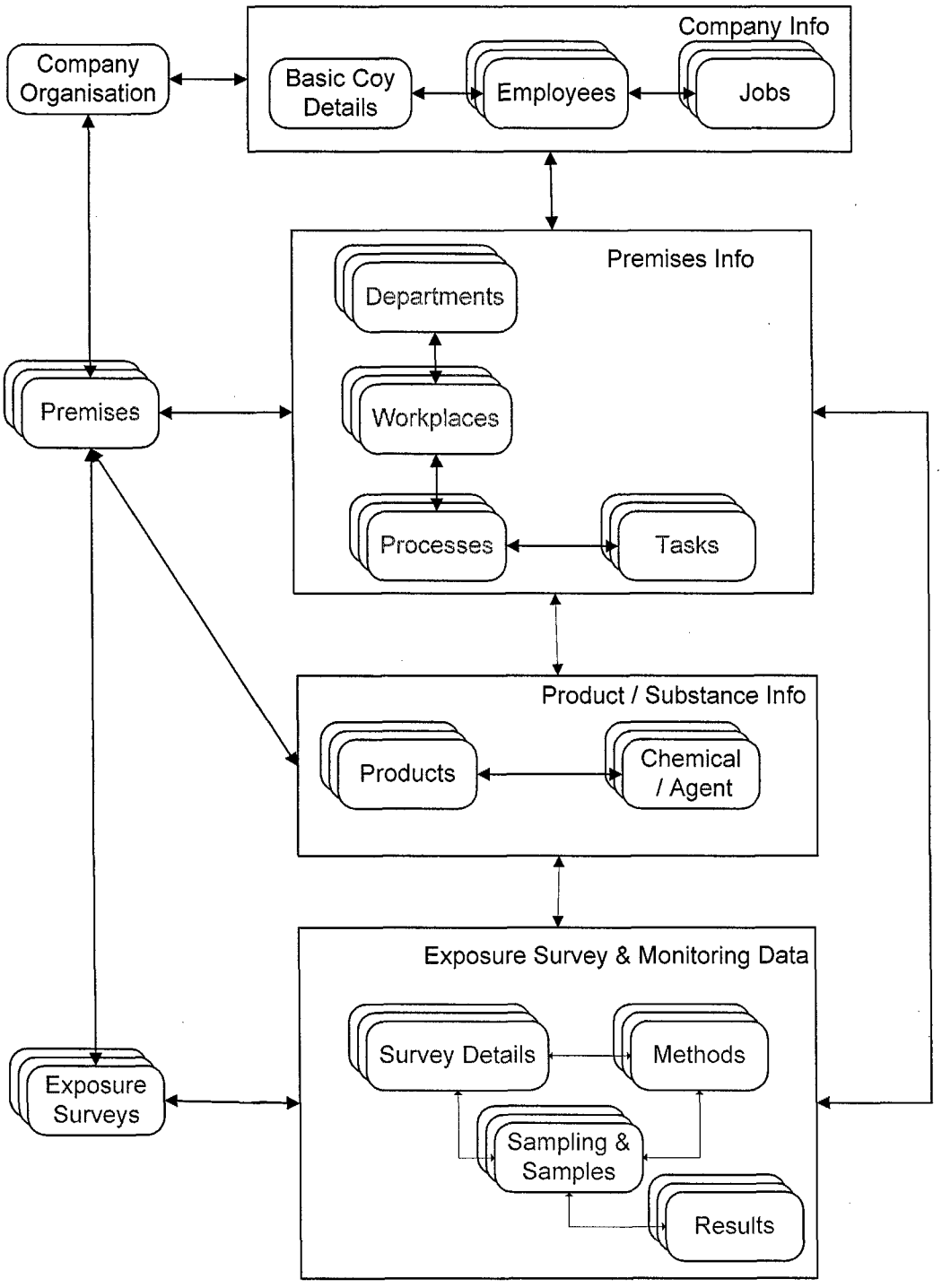


Figure 1: Key database elements

ManganEx Database: Idealised Schematic Data Model for
Company & Premises Level View - Key Entities of Interest and Relationships

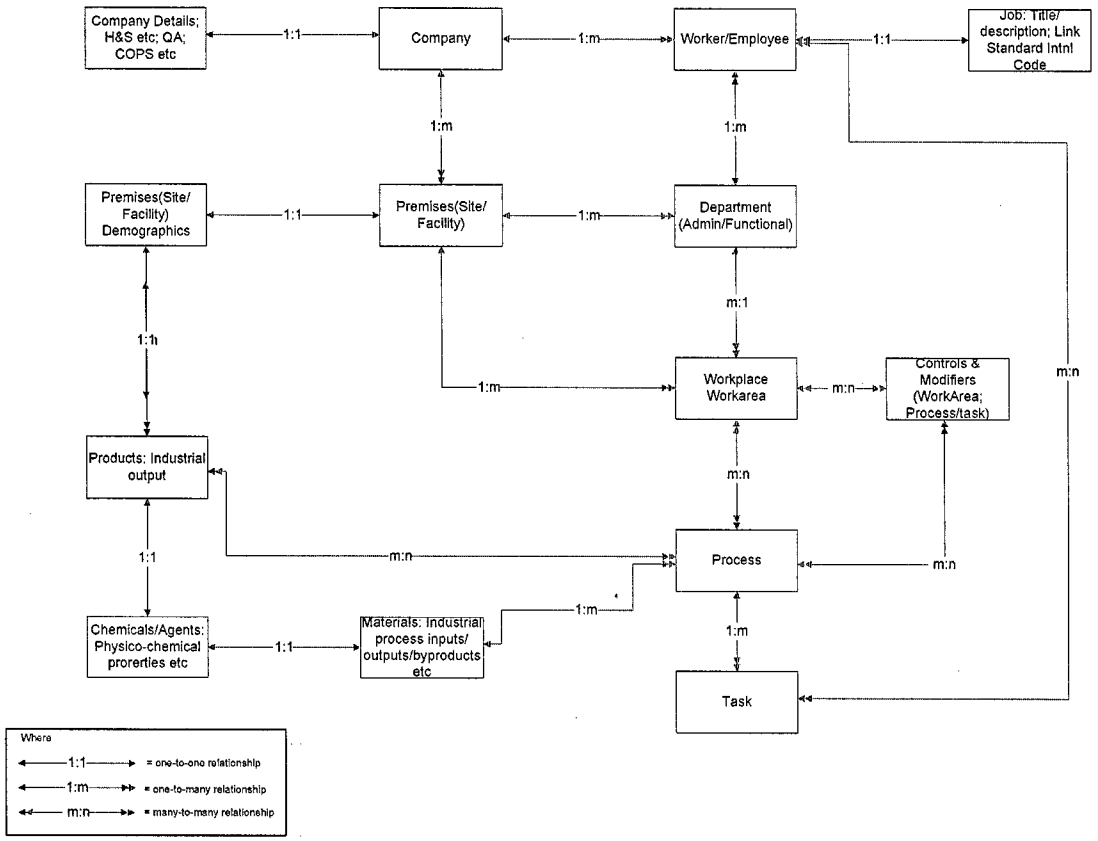


Figure 2: Company/Premises level - Entity relationship diagram

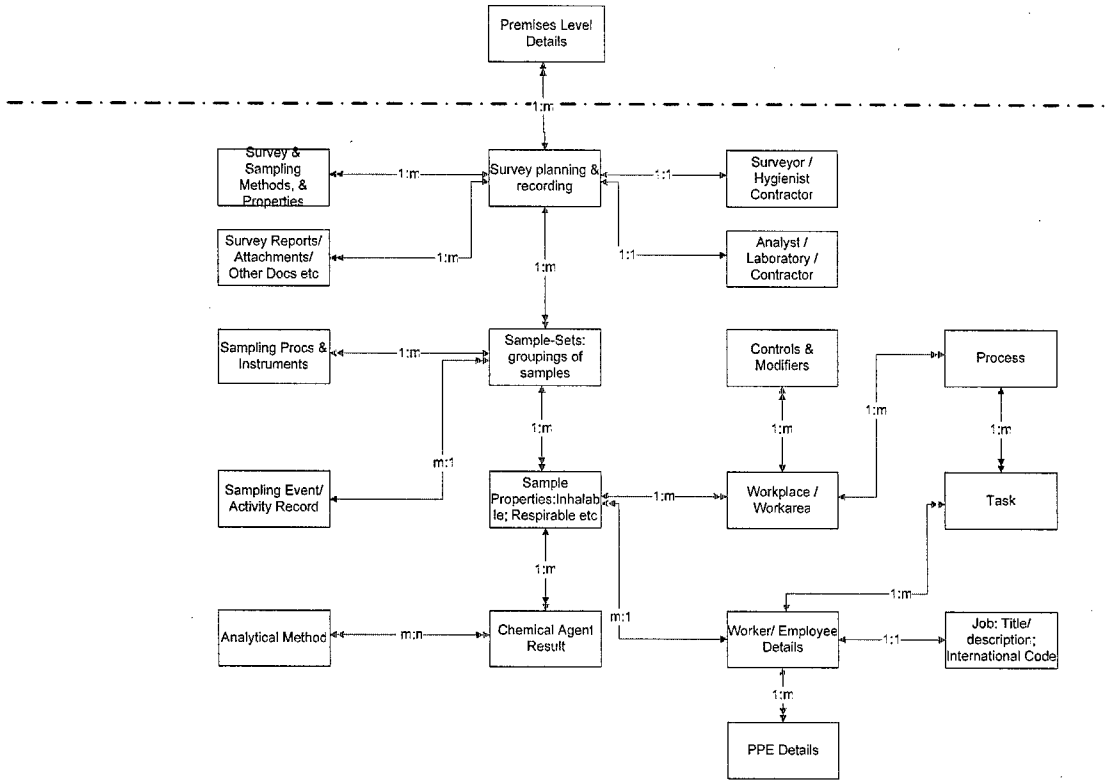


Figure 3: Survey Level - Entity relationship diagram

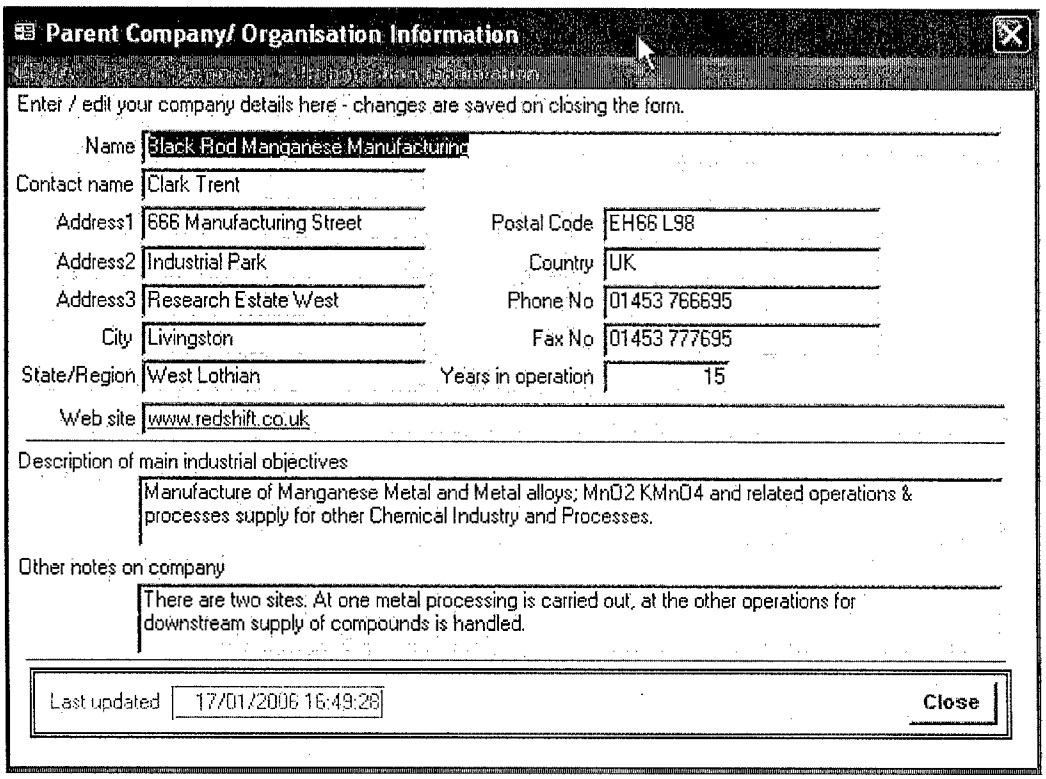


Figure 4: Company level data management screenshot

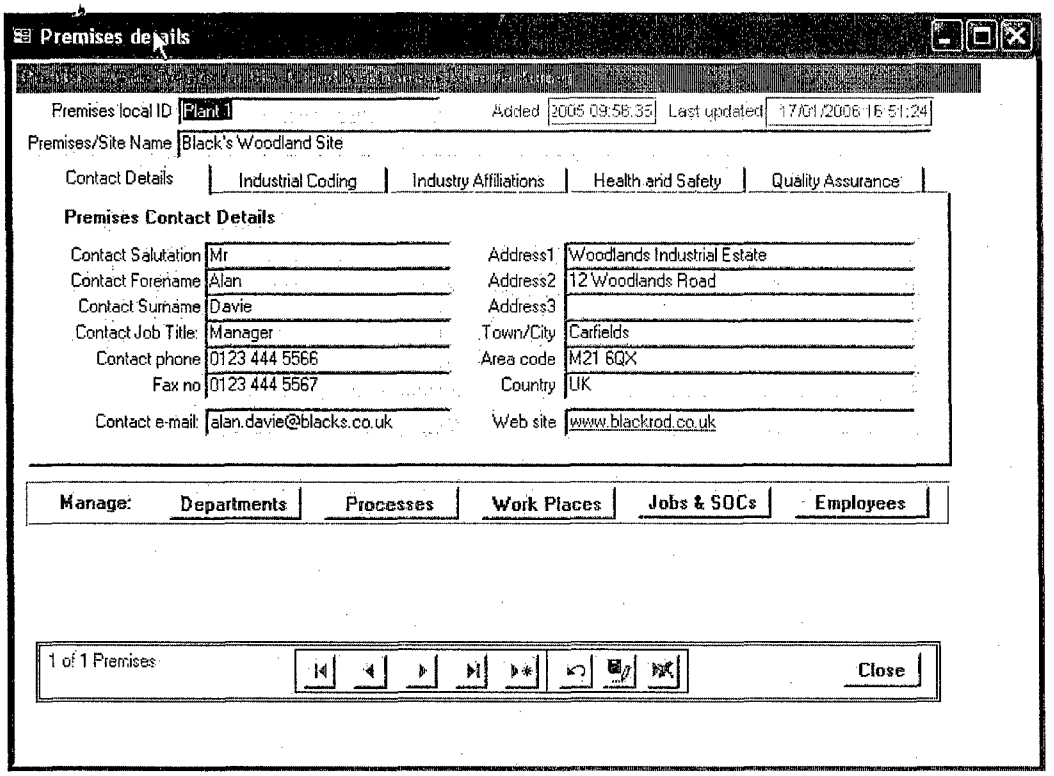


Figure 5: Company level data management screenshot

Key Research Accomplishments

There are no key research accomplishments at this stage.

Reportable Outcomes

At this early stage in the project there are no reportable outcomes.

Conclusions

It is not appropriate to draw any conclusions at this stage of the work.

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CORE 2

Biomarkers of Early Onset of Manganese Neurotoxicities among Occupationally Exposed Chinese Workers

Wei Zheng, Ph.D.
Purdue University School of Health Sciences
West Lafayette, IN 47907

ABSTRACT:
Exposure to Mn occurs in both civilian and military settings. Currently, there is no practical method or the biomarker available for early diagnosis of Mn-induced neurodegenerative damage. We proposed a cross sectional design to evaluate the associations between airborne Mn levels and biomarkers of exposure (the concentrations of Mn, Fe, and Fe regulatory proteins) in biological matrices, and between the concentrations of biomarkers and the early signs of neurological deficits. The cohort is located in Zunyi City in China, with industries involving Mn mining, refining, smelting, processing, and ferroalloy production and with the existing Mn intoxication cases. We propose to recruit a total of 300 subjects into this project. The IRB protocol has been officially approved by Zunyi Medical College (ZMC) in 20 Aug 2005, by Purdue Univ on 20 Sep 2005, and conditionally approved by the U.S. AMRMC Human Subjects Research Review Board on 22 November 2005. The Human Research Assurance Number of the ZMC has been awarded by U.S. DHHS on 13 Jan 2006. Once the IRB is finally approved (expected in Feb AMRMC IRB meeting), the study will be initiated immediately. A site visit to the ZMC has been scheduled between April 17-20, 2006 by Drs. Aschner and Zheng aiming to monitor the progress, control the quality, and perform technical guidance.

Introduction

The early onset of Mn intoxication is usually subtle and progressive. The initial signs may be categorized as the nonspecific neurological manifestations, psychiatric symptoms, and extrapyramidal signs. The exposed workers may complaint asthenia, anorexia, apathy, insomnia or drowsiness, malaise, somnolence, or diminished libido or impotence. Psychiatric symptoms are more specifically indicative of Mn toxicity, including disorientation, emotional instability, compulsive acts, hallucinations, illusions, delusions, and slurring and stuttering speech with diminished voice. These are followed by selective extrapyramidal disorders such as imbalance in walking or on arising, finger coordination, and tremor.

Since Mn induced neuronal damage is irreversible, the early diagnosis becomes crucial for prevention of Mn toxicity in occupational and environmental exposure scenarios. Based on a recent study on 97 welders in Beijing, China, Dr. Zheng and his colleagues in China has found that serum concentrations of ferritin and transferrin were increased among welders, while serum transferrin receptor levels were significantly decreased in comparison to controls. Moreover, this group found that serum transferrin receptor levels were inversely associated with serum manganese concentrations ($p<0.05$). Thus, these iron regulatory proteins along with Fe itself may serve as the potential useful biomarker for early diagnosis of Mn toxicity.

We propose a three-phase, three-year, cross-sectional study to test the hypothesis that occupational exposure to airborne Mn is associated with health disorders among exposed workers in a time-dose dependent manner. More specifically, we aim to see if airborne Mn levels are positively correlated with levels of Mn in blood, urine, saliva, or hair, and Fe or Fe regulatory proteins in serum, one of which can be used as the biomarker to assess Mn exposure. In addition, we aim to study whether Mn concentrations in biological matrices (blood, urine, or hair) are associated with early signs of health disorders among exposed workers.

In Phase I, the principal task is to further characterize the study sites and to conduct exposure assessment in the environment from which the study subjects will be recruited. During this period, the instruments for air sampling, questionnaires for epidemiological study, documents for data storage and methodology for laboratory assays (AAS) for Mn, etc., shall be fully prepared or developed.

The Phase II aims to study the biological outcomes of exposure. We will collect biological samples, conduct physical examinations, and determine Mn and biomarker concentrations. Biological samples from all workers will be obtained at the time of physical examination within 10-12 months. The time frame for data collection will be approximately 12 months and the lab analyses will take longer time.

In Phase III, we will put much our effort on statistical analysis to draw the conclusions on our hypotheses. We estimate a 9-12 month period, for we may revisit some of the sampling spots or subjects to verify the data.

Body

1. Human study logistics

The IRB protocol entitled "Biomarkers of Early Onset of Manganese Neurotoxicities among Occupationally Exposed Chinese Workers" (Ref#04-655) was originally approved by the Committee on the Use of Human Research Subjects, Institutional Review Board of Purdue University, on 29 October 2004. It was re-approved on 19 September 2005 at Purdue. The IRB protocol with the same title in Chinese was officially approved by Zunyi Medical College (ZMC) in 20 Aug 2005.

The initial application for IRB approval was sent to the Human Subjects Research Review Board (HSRRB) of the U.S. Army Medical Research and Materiel Command (AMRMC) on 26 August 2005. The application was suggested for full review and subsequently reviewed by AMRMC HSRRB on 12 October 2005. The revision was conditionally approved by the Committee on 22 November 2005. During the same time period, the ZMC has filed its IRB Committee and human study protocol with the Office for Human Research Protections (OHRP) on 23 September 2005 and obtained an IORG number (#0004280) on 12 October 2005. With all required documents, the human research assurance number has been approved and granted to the ZMC by U.S. DHHS on 13 Jan 2006. This number along with other required modifications to the original consent form has been sent to the AMRMC HSRRB for consideration of final approval, which is expected in February 2006.

Once the IRB is finally approved, the study will be initiated immediately.

2. Air sampling in selected locations

According to the national standard set out by the Chinese Ministry of Public Health (TJ36-79), the maximum allowable concentration (MAC) of Mn in the work place is 0.2 mg/m^3. Thus, the persons who work in Mn manufacturers with air Mn levels that are more than 10 fold higher than the MAC value will be defined as the **high** exposed subjects ($\geq$ 2 mg/m^3); the persons who work in the same factories and are exposed to air Mn levels above 0.1 mg/m^3 but less than 2 mg/m^3 are defined as the **moderate** exposed subjects; those who are not directly engaged in Mn production with the exposure level less than 0.1 mg/m^3 are defined as the **control** or minimal exposure subjects (the ambient Mn level in Zunyi area based on the past study is less than 0.5 $\mu g/m^3$). Several candidate study locations for all three groups have been selected with the stationary air samplers; however, the final decision as to which ones will be used in this study should be made by the data collected from the personal air samplers. This will be done once the IRB study protocol is approved.

3. Trip to Zunyi to consolidate collaboration in April 2005

Drs. Zheng (team leader and neurotoxicologist), Rosenthal (expert in exposure assessment), and McGlothlin (expert in industrial hygiene and epidemiology) at Purdue and Dr. Jie Liu of NIH/NCI (expert in bioassays) visited Zunyi, China between April 4-9, 2005. The purpose of this trip was (1) to consolidate working relationship with Chinese counterpart, (2) to establish the direct communication channels between the investigators from the US and China, (3) to clearly define and assign the responsibility to each researcher in this multination team, (4) to train the researchers on the site for how to use the equipment we brought to ZMC, and (5) to discover the potential problems and to solve them on the site. The visit resulted in a signed Research Agreement between Purdue University and ZMC.

4. Site Visit by the Program Director and the P.I. in April 2006

In order to ensure the highest quality of this research, a site visit to the ZMC will be conducted and has been scheduled between April 17-20, 2006. Dr. Michael Aschner, the Program Director, Dr. Wei Zheng, the PI of this project, and Mr. Dallas Cowan, doctoral student in Zheng group, will form the site visit team aiming to perform the following tasks:

1) for Dr. Aschner to meet the research team and to oversee the progress (Aschner, Zheng)
2) to examine if the human research conduct follows the IRB and other protocols (Zheng, Aschner)
3) to conduct neurobehavioral testing on the subjects (Zheng, Cowan)
4) to monitor laboratory experiments and assays (Zheng, Cowan)
5) to bring some biological samples back to the US for quality control (Cowan)
6) to discuss the exchange scholar for training propose (Aschner, Zheng)

Key Research Accomplishments

- IRB protocol has been approved by Purdue and ZMC committees and awaits for the final approval by the DoD committee.

- The ZMC was granted an Assurance number by the U.S. Department of Human Health Services, one of the six in OHPR official website.

- The potential human study sites have been screened and selected.

- A formal collaboration relationship has been established between the U.S. investigators and their Chinese counterpart.

- A working site visit has been scheduled to monitor the progress and quality before the full scale of research is embarked.

Reportable Outcomes

- Abstracts already presented:

Not applicable

- Current faculty receiving support from the grant:
  - Wei Zheng, PhD
  - Frank Rosenthal, PhD

- Current students receiving training from participation on projects related to this grant:
  - Dallas Cowan

Conclusions

While the project has not reached its full-swing stage, we have successfully established the working relationship between the US and Chinese investigators. Under the guidance of IRB Committees in each institute and particularly with the AMRMC, we have thoroughly modified our human study protocol to meet the highest standard in human research. We have also pinpointed the candidate study sites for formal research. In addition, the preliminary training has been conducted and the communication channel has been established. In all, we are in a good shape to initiate research once our IRB is formally approved in Feb 2006.

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Not applicable

CORE 3

Exposure to Welding Fume and Parkinson's Disease: a Feasibility Study.

Jouni Jaakkola, M.D., Ph.D, D.Sc
Craig A. Jackson, Ph.D.
Grant McMillan, M.D.
Sean Semple, Ph.D.
David Nicholl, M.D.

Institute of Occupational & Environmental Medicine
University of Birmingham, UK

ABSTRACT:
This study is a feasibility study to inform the planning and design of a larger-scale study phase 2) anticipated to begin in 2007, depending on the outcomes of the feasibility stage. The secondary outcomes of the study are intended to refine the number of methodological options option at present for the phase 2 study, specifically in three areas: Case definition of "manganism"; participant selection; and Retrospective Exposure assessment. Steel welders probably constitute the largest occupational group exposed to manganese and oxides from cutting and arc welding of steel. The specific neurological disorder attributed to excess absorption of manganese is "manganism" - slowly progressive deterioration of well-being coupled with specific disturbances of mood and muscle function, bearing a marked similarity to Parkinson's Disease (PD) and is referred to as "Manganese-induced Parkinsonism". A small case-control study of steel welders with idiopathic PD (Racette et al. 2001) showed no clinical differences between them and the typical PD population, but their disease was distinguished by a statistically significant younger age of onset. This finding promoted the hypothesis that employment as a welder may be a risk factor for PD, accelerating or triggering the onset of the disease. A case-referent study represents the most appropriate means of investigating this hypothesis.

I. A significantly higher
proportion of those having PD have been steel welders, or exposed to manganese-containing metal fumes, than those in a matched control group (age, sex, education) who do not have PD.

II. Within those having PD, the age of
onset is lower among those who have been occupationally exposed to manganese than those who have not.

Introduction

Caveat

This study is a feasibility study to inform the planning and design of a larger-scale study (phase 2) anticipated to begin in 2007, depending on the outcomes of the feasibility stage. The commencement of this study was delayed by the retirement of the Principal Investigator at the time, Dr Anne Spurgeon, and then further delayed by the University of Birmingham failing to replace Dr Spurgeon with another suitably qualified psychologist to run the Feasibility Study. Another psychologist was identified and appointed to the study in November 2005, and this report is therefore an account of all the material and procedures performed since the recommencement of the Feasibility Study in November 2005. The secondary outcomes of the study are intended to refine the number of methodological options at present for the phase 2 study, specifically in three areas: Case definition; Control selection; and Retrospective Exposure assessment, and the key areas are outlined below.

Case-definition

The reliability and validity of the definition of a "case" is central to a case-referent study. However, the diagnosis of idiopathic PD is not straightforward in the absence of biological markers for ante-mortem diagnosis, and this currently depends on the presence and progression of clinical features. Misdiagnosis has been shown to be common (particularly in the early stages of the disease) and such misdiagnosis is a factor which has been suggested as a limitation of several epidemiological studies investigating the causes of PD (Litvan *et al.* 2003). A number of sets of diagnostic criteria have been proposed, including:

(i) Presence of at least two "cardinal signs"

(ii) Parkinson's Disease Staging Scale (Hoehn and Yahr, 1998).

(iii) UK Parkinson's Disease Society Brain Bank (Hughes *et al.* 1992).

Studies have been carried out to examine the concordance between diagnoses using these different scales and those of neurologists. It is proposed that all these criteria be used in selecting participants with PD for this study, in order to determine the most defensible approach to case definition and to design a system whereby the validity of case definition can be assessed and maintained during the course of the study.

Control selection

The source of suitable matched controls will depend largely on the method of case identification (by consultant Neurologists). However the recruitment of controls, notably in respect of their agreement to participate, is known to be problematic. Source options include either the "buddy" system where cases

identify friends who meet certain criteria, or selection of other patients attending other out-patient clinics.

Retrospective Exposure assessment

A method for the retrospective assessment of exposure, which combines the advantages of a job exposure matrix with questionnaire data, has been developed by Semple et al. (2004), investigating a range of possible occupational, environmental and genetic risk factors for PD. The methodology shows promise in terms of application to the current proposed study. However, although validation of the method has been carried out in respect of exposure to organic solvents, it awaits validation in terms of manganese exposure. Semple (Department of Environmental and Occupational Medicine at the University of Aberdeen) will carry out such a validation on a sample of manganese-exposed workers, specifically in terms of the concordance between exposure estimations obtained using this method and those obtained using detailed assessments of occupational hygienists. This work will NOT involve the participants in this feasibility study, but rather a group of workers using manganese who volunteer to have their exposures monitored and compared with their estimated retrospective exposures.

Progress

Each entry listed below is a development in the preparation and planning of the Feasibility Study, since the study was actively re-started by Birmingham University in November 2005

12th Nov 2005

Antecedent Birmingham University appoint Craig Jackson as lead researcher to commence the study

22nd Nov 2005

Antecedent Contact made by International Manganese Institute (Anne Tremblay – General Secretary).
Outcome IMI wish to proceed with Feasibility Study as soon as possible.
Outcome IMI satisfied for Craig Jackson to act with major responsibility for the Feasibility Study.

27th Nov 2005

Antecedent Discussion with research team members over methodology and protocol.
Outcome Methodology and protocol finalized.
Outcome Decide to gain participation of Dr Grant McMillan as Project Consultant.

1st Dec 2005

Antecedent Correspondence with USAMRMC (Donna Ferrandino – Human Subjects Protection Scientist).
Outcome Discuss procedure for Feasibility Study gaining USA MRMC ethical approval.

7th Dec 2005

Antecedent Met with Dr Grant McMillan.
Outcome Dr McMillan agreed to participate in the study as Research Consultant.

12th Dec 2005

Antecedent Re-costing of the Feasibility Study.
Outcome Study can be conducted by Birmingham University for the agreed amount.

21st Dec 2005

Antecedent Inform USAMRMC of UK rules and procedures for gaining ethical approval for patient studies.

29th Dec 2005

Antecedent USAMRMC agree that UK research ethical approval should be sought in first instance.
Outcome Birmingham university to seek approval from local research ethics committee.
Outcome Craig Jackson agrees to keep USAMRMC informed of ethical application and decision.

12th Jan 2006

Antecedent Craig Jackson contacts Miki Aschner re: minor changes to study e.g. PI name.
Outcome Minor changes acceptable without any amendments to contract needed.

13th Jan 2006

Antecedent Discussions with project team re: PD patient and control recruitment and access.
Outcome Access to PD patients in a local movement disorder clinic run once a week is agreed.

15th Jan 2006

Antecedent Entering objectives, theory, methodology and data collection process in ethical application form.
Outcome Ethical application will be completed and submitted by Jan 31st 2006.

14th Mar 2006
Antecedent South Birmingham Research Ethics Committee panel convene and review application
Outcome Ethics committee will notify Craig Jackson of decision e.g. approval or amend.

Key Accomplishments

1) Replacement of Anne Spurgeon with new Principal Investigator & Lead Researcher

2) Securing integrity of the research team remained while replacement Principal Investigator & Lead Researchers were being sought.

3) Re-costing was achieved within budget after allocation of replacement Principal Investigator & Lead Researcher.

4) Ensuring access to a clinical population of males with Parkinson's disease likely to contain a suitably high number of individuals with previous occupational manganese exposures.

Targets

Forthcoming targets essential to the commencement and completion of the Feasibility Study include:

1) Securing ethical approval from the South Birmingham Research Ethics Committee at the earliest opportunity.

2) Informing the Human Subjects Protection Scientist at the USAMRMC once ethical approval has been secured, and if any changes will be required to the study methodology in order to secure such approval.

3) Ensuring contract exchanges and release of research funds from Vanderbilt University once ethical approval is secured.

4) Perfecting patient recruitment techniques (poster & leaflet campaigns; increase awareness of local consultant neurologists) to ensure best recruitment rates from the local weekly movement disorder clinic, and a sample of local age/sex matched control participants.

5) Performing sample size calculation on initial data in the Feasibility Study in order to ensure the available sample size is powerful enough to answer the two research hypotheses. If initial sample calculations indicate this is not so, the South Birmingham Research Ethics Committee will be informed of the intention to change sample size. The USAMRMC will also be informed.

Conclusions

Within three months of this Feasibility Study having two new lead members attached to it, as well as retaining the high calibre of collaborators already involved in the project, the research team are now in a position to complete the application to secure ethical approval from the local governing body. This study has acquired a new energy and vigor, evident in the recent quick progression of the planning of the project, and if maintained, this study should provide all of the required information to enable a decision concerning the future conduct of any full-scale investigation into occupational manganese exposure and Parkinson's disease.

Reportable Outcomes

This study is not at a stage where any outcomes can be reported.

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CORE 4

Provision of Research Awareness Services

Levy, Leonard, Ph.D.
Holmes, Philip, B.Sc .
Harris, Robert, MSc

Institute of Environment and Health
Cranfield Univeristy
Silsoe, Bedfordshire UK MK45 4DT

ABSTRACT:
The extent of research conducted globally on the effect of manganese on human health is extensive (e.g. >500 papers published in 2002 alone), posing a considerable challenge to researchers to keep up to date with recent progress in understanding. To assist researchers and other interested stakeholders (such as regulators and public/occupational health professionals), the IEH is developing, in conjunction with the International Manganese Institute (see Core 7), a series of knowledge-based tools that capture and synthesis data into readily accessible forms. The services to be provided by IEH comprises a database of recent and ongoing research projects, a quarterly update service of relevant recent publications, and an annual overview report to summarizing key research findings and achievements over the period: These will be delivered through an open-access web-site to interested parties. Initial planning and development of the website and database has been completed, and work is progressing on the other aspects.

Introduction

The extent of research conducted in recent years on the nature and pattern of human exposure to manganese, and its potential to affect human health is very extensive with, for example, over 500 English-language papers being published in 2002 alone. Such a volume of publications poses a considerable challenge to researchers and other stakeholders if they wish to keep up to date with recent findings and development of current understanding. However, just such an awareness is essential for researchers if needless duplication of effort is to be avoided, and is similarly of great importance to regulators and research funders alike if improved application of current scientific understanding and the precise targeting of funds towards the remaining critical gaps in the knowledge base, are to be achieved.

In order to assist researchers and these other interested stakeholders (such as regulators and public/occupational health professionals), the Institute of Environment and Health (IEH) is developing a series of knowledge-based tools, in conjunction with colleagues at the International Manganese Institute (see Core 7), to provide all interested stakeholders with a readily accessible, comprehensive series of research awareness tools intended to capture and synthesis all available data into readily accessible forms.

Specifically, the services to be provided by IEH comprise:

- a database of recent and ongoing research projects that address the relationship between exposure to manganese and human health;
- provision of a quarterly update service that identifies published papers of relevance; and
- production of annual overview reports (based upon the output of the quarterly awareness service and the research database) that identify and summarize the key research findings and achievements over the period, and identifies the profile of ongoing research activities over the period.

These services will be delivered to stakeholders freely through an open-access web site, which will also incorporate other relevant information that will be developed by IMnI under Core 7.

Body

In the short period of time since the signing of the contract between Vanderbilt University and Cranfield University on the 10 November 2005, the project has already gained considerably momentum.

A Project Initiation meeting was held at the Royal College of Physicians in London, UK on the 22 November 2005 at which the IEH project team met with representatives of IMnI to discuss and agree the required approach and outputs from this project and the associates activity Core 7 (Coordination of the Mn Health Research Programme Steering Committee and Administration of the Research Activity Awareness Services). IEH have since completed a number of essential initial steps including:

- provision of text for inclusion on the website to describe the nature and purpose of the various information types that will be provided by IEH;
- development of a customised questionnaire to obtain information on researchers and their projects for subsequent inclusion on the database; and
- analysis of design requirements and functionality for the model of the database (see Appendix 1).

Programming for the database is now in progress, with the data entry functions being scheduled for completion before the end of January. In addition, a structured search strategy (to be employed to produce the quarterly awareness searches and to assist in the identification of researchers to whom questionnaires will be sent to collect information for population of the database) is in development.

In summary, development of the database and associated services are progressing well, and it anticipated that the launch of the website - including information on the database and access to a downloadable questionnaire for submission of information – will be achieved by 24th January 2006. This will provide the platform for the IEH database and research awareness services that will be produced by IEH during the remaining period of the current contract. In

subsequent years, efforts will be focused on ongoing provision of current awareness services and on substantially increasing the extent of information held on the database.

Key Research Accomplishments

Not applicable – key milestones will be achieved during the remaining period of the current contract (i.e. to 30 June 2006).

Reportable Outcomes

No outcomes have as yet been reported in the open literature.

Conclusions

The work on developing the database and associated services is progressing well, and it is anticipated that the launch of the website - including information on the database and access to a downloadable questionnaire for submission of information – will be achieved by 24th January 2006. This will provide a platform for the proposed database and research awareness services that will be produced by IEH during the remaining period of the current contract. In subsequent years, it is anticipated that the extent of information held on the database will increase further so that it becomes an internationally-essential gateway for information on research into the health effects of manganese.

References

Not applicable.

Appendices

Appendix 1: Proposed functionality of DOGRAM database

Introduction

Development of the database involves three distinct technical tasks:

- designing a Microsoft SQL Server 2000 database to store the data;
- developing a data entry system using Microsoft Access 2003; and
- developing the website using ASP.Net.

The database structure will be developed around three core tables of information:

- Project details;
- Individual details; and

- Organisation details.

The following sections describe the contents of the core tables, and the related data associated with each table. Descriptions focus on the ***type*** of information being collected rather than the technical format of the data.

1. Projects

Specific data incorporated in the Projects table will include:

- Project title
- Start month
- Start year
- End month
- End year
- Revised start month
- Revised start year
- Revised end month
- Revised end year
- Comments about revised dates – i.e. reasons for delay
- Project status (yet to start/ongoing/completed)
- Project value (if supplied by the principal investigator)
- Source (s) of funding

The Projects table will be related to other data through the following associations:

- A project will have researchers associated with it and they will be classified by their status on the project e.g. project leader, research team member, collaborator, etc. Where an individual's name is not provided, the research organisation only will be associated with the project.
- A project will have funding details associated with it (if provided by the project leader). These will include project value, funding bodies (main funders and co-funders will be accommodated) and contact person (with address/contact details) within the funding body.
- A project will be key-worded using terms from a database-specific *n*-level hierarchical thesaurus. Each project will therefore be associated with keywords from one of the *n*-levels of the thesaurus categories, as appropriate.
- A project will have publication details associated with it (if provided by the project leader). These will include publication title, authors, bibliographic details, abstract (subject to any copyright limitations) and a project web address (if available).

2. Individuals

Specific data incorporated in the Individuals table will include:

- Title (Prof, Dr, etc)
- First name
- Surname
- Other information

The Individuals table will be related to other data through the following associations:

- An individual will work for (or be associated) with an organisation. If provided, they will have direct contact details in conjunction with the organisation contact details. Independent consultants will also be included.
- An individual will have a specific role on a project e.g. project leader, research team member, collaborator, etc.

3. Organisations

Specific data incorporated in the Organisations table will include:

- Organisation name e.g. University, Company name
- Department/Section/Institute e.g. Institute of Environment and Health; Department of Chemistry
- Building

- Street
- District
- PO BOX
- Town
- County
- Post/Zip code
- Country
- Telephone/tel/fax/email/WWW details are for the organisation (direct contact details for the individual will be included separately)

The Organisations table will be related to other data through the following associations:

- A research organisation can employ one or more individuals who have a specific role on a project, but only one will be the main project contact (who need not necessarily be the project leader (since they may have delegated the role to another member of the research team).
- A funding organisation can fund a project.

CORE 5

Cellular Mechanisms Involved in the Uptake and Delivery of Inhaled Manganese Via the Olfactory Nerve

Dorman, David, C., D.V.M., Ph.D.

CIIT Centers for Health Research
6 Davis Drive
Research Triangle Park, NC 27709

ABSTRACT:
Neurotoxicity is a significant public health concern associated with manganese (Mn) inhalation. Inhaled Mn is deposited in the olfactory epithelium and can be transported directly to the mammalian brain via the olfactory nerve. We hypothesize that the divalent metal transporter (DMT-1) plays a role in the initial uptake of inhaled Mn by therat olfactory epithelium. Progress completed during the first funding year has included confirmation of DMT-1 has slightly higher expression in the rat olfactory epithelium when compared to the respiratory epithelium (1.5-fold, $p < 0.05$). We also developed anesthesia methods needed to perform the viral delivery methods. Another objective was to complete the pilot transfection studies using lentiviruses expressing green fluorescent protein (GFP) or Lac-Z. Unexpectedly, autofluorescence of the nasal epithelium confounded studies with the GFP marker. DMT-1 transfection and 54Mn inhalation studies (Aims 2 and 3) will be completed in the upcoming year.

Introduction

Neurotoxicity is a significant public health concern linked with high-dose manganese inhalation. People at risk for manganese neurotoxicity include welders and other workers involved in metal smelting operations, steel production, and foundries. A critical step in the pathogenesis of manganese neurotoxicity is the initial accumulation of the metal in the brain. Unlike many xenobiotics, inhaled manganese deposited in the olfactory epithelium can be transported directly to the brain via the olfactory nerve. Direct nose-to-brain (i.e., olfactory) delivery of manganese has been observed in multiple animal species, including nonhuman primates, suggesting that this route of delivery could occur in people. Few studies have evaluated the mechanism by which manganese is absorbed by the olfactory epithelium and then ultimately transported. We hypothesize that the divalent metal transporter (DMT-1) plays a role in the initial uptake of inhaled manganese by the rat olfactory epithelium. We have chosen the rat because the olfactory transport of manganese has been well studied in this species. We hypothesize that DMT-1 plays a role in the initial uptake of inhaled manganese by the rat olfactory epithelium. We expect to show that brain levels of manganese (as ^{54}Mn) will be decreased on the side of the head with degraded DMT-1 mRNA.

Objective 1: Develop adenoviral and lentiviral shRNA vectors for DMT-1. Completion of this study will require us to alter DMT-1 expression in the rat nose. Our initial pilot studies examined whether these viral vectors can be used to transduce the rat olfactory epithelium with a reporter (e.g., green fluorescent protein [GFP] or β-galactosidase [LacZ]). Rats (n = 3/vector/time point) will be killed and nasal tissues collected in order to determine transduction efficiency. Completion of the pilot studies was delayed considerably due to the following reasons: (a) anesthesia methods we had previously used for nasal instillation studies were not compatible with the longer anesthesia times required to complete the slow delivery of the modified viruses. Moreover, auto-fluorescence present within the rat nasal epithelium interfered with our ability to detect the presence of the GFP following viral transduction. This required us to switch our approach from GFP to LacZ as the reporter system. Finally, transfection rates associated with the viruses were lower than anticipated based on work published by other investigators using mouse models. Each of these technical limitations has been addressed; however, they have led to significant delays in our ability to complete this phase of the project. We are in the process of adapting both of the viral vectors to contain short hairpin RNAs (shRNAs) for DMT-1.

Objective 2: Deliver viral particles containing the shRNAs for DMT-1 to rat olfactory epithelium and demonstrate a reduction of DMT-1 mRNA and/or protein. Once the viral vectors have been developed then we will administer virions to anesthetized rats by intranasal instillation (right nostril only). Infected rats will be killed at 1, 2, or 3 days after infection in order to confirm changes in DMT-1 mRNA expression or protein levels in the nasal mucosa. This activity will occur in year 02.

Objective 3: Determine whether reduced DMT-1 expression alters the delivery of inhaled manganese to the rat brain. Once we confirm that treatment with the shRNAs alters DMT-1 in the rat olfactory epithelium, we will proceed with inhalation studies with radiolabeled manganese chloride ($^{54}MnCl_2$). These inhalation studies will be performed on rats that have been pre-treated with a viral vector containing shRNAs against DMT-1 mRNA. Timing of the manganese exposure will occur when DMT-1 expression is altered. Rats will be exposed to $^{54}MnCl_2$ for 90 minutes and killed (by lethal barbiturate injection) 1, 2, 4, or 8 days after inhalation exposure. Brain and nasal tissues will be harvested and evaluated for ^{54}Mn levels. We expect to show that brain levels of manganese (as ^{54}Mn) will be decreased on the side of the head with decreased DMT-1 mRNA. This activity will occur in year 02.

Body

Olfactory epithelial DMT-1 expression. The following work was completed using funding available from a separate grant provided to Dr. Dorman's laboratory by the American Chemistry Council. This research is directly related to the MHRP project in the following ways: (a) developed epithelial cell isolation methods needed for isolation of DMT1 mRNA and protein; and (b) confirmed DMT1 mRNA expression in the rat nasal epithelium. Naïve 10-week old male and female CD rats were deeply anesthetized with sodium pentobarbital (60 mg/kg) and exsanguinated. Nasal samples, consisting of the nasal septum, and the naso-, maxillo-, and ethmoid turbinates, were dissected free from the nasal cavity. The nasal septum, and naso- and maxillo- turbinates were used as a source for respiratory epithelium and the ethmoid turbinates were used as source for olfactory epithelial cells. The respiratory and olfactory epithelium was manually dissected directly from the boney and cartilaginous nasal turbinates and septum. The absence of epithelium from turbinate and septal tissues was confirmed via histology. Nasal respiratory and olfactory epithelial

samples were homogenized in TRIZOL® Reagent, 1 ml /50-100 mg of tissue, and separated by centrifugation in 0.2 ml of chloroform /1 ml of lysis reagent using heavy Phase Lock Gel™ tubes. RNA was precipitated from the aqueous phase using isopropyl alcohol and pelleted with centrifugation. The pellet was washed and applied to the QIAGEN RNeasy Mini kit column. RNA was digested free of DNase on-column using the QIAGEN RNase-Free DNase Set, washed in buffer, and eluted with RNase-free water. Quality of the RNA was verified with standard spectrophotometric analysis and gel electrophoresis using the Agilent 2100 Bioanalyzer. Preparation of the probe and hybridization to the microarray was performed by CIIT's Gene Expression Core. Double-stranded cDNA was synthesized from RNA samples using an oligo-dT24-T7 from 5 µg of total RNA/sample. Synthesized cDNA template was transcribed to biotin-labeled cRNA using the GeneChip® IVT Labeling Kit. Fifteen µg of labeled cRNA was fragmented and hybridized to Affymetrix Rat Genome 430 2.0 arrays in the Hybridization Oven 640 for 16 hours at 45°C. After hybridization, arrays were washed using the GeneChip® Fluidics Station 450 and scanned with the GeneChip® Scanner 3000.

Expression data were preprocessed using RMA with a log base 2 (log2) transformation. Statistical analysis of the microarray data was performed in R using the affylmGUI package. To identify genes with significant changes in expression between tissue types and gender, data were analyzed using a linear model with contrasts between male and female and respiratory and olfactory epithelium. Probability values were adjusted for multiple comparisons using a false discovery rate of 1% (FDR = 0.01). Genes identified as statistically significant were subject to an additional filter by selecting only those genes that exhibited a $\geq$ 2-fold change. Analysis of gene ontology (GO) categories was performed using Onto-Express. Affymetrix probe set identifiers for the genes of interest were uploaded to the Onto-Express web site (http://vortex.cs.wayne.edu/index.htm) and analyzed based on the Affymetrix U133A_2 reference list. A hypergeometric test was performed to identify GO categories with significantly enriched gene numbers with *p*-values corrected using a false discovery rate of 5% (FDR = 0.05). The resulting list of GO categories was further refined by removing categories containing > 4 genes.

DMT-1 has slightly higher expression in the rat olfactory epithelium when compared to the respiratory epithelium (1.5-fold, $p < 0.05$). In the upcoming year and using MHRP funding, the project will develop quantitative PCR and protein analysis methods (Western blots) for DMT-1 in the olfactory epithelium from naïve and viral vector-treated rats. These methods will be used to confirm that treatment with viral vectors that express inhibitory mRNAs results in reduced DMT1 expression and protein levels.

Vector production: Prior to performing shRNA knockdown of DMT1, we first assessed the tropism and infectivity of adenovirus and lentivirus in the nasal epithelium using a constitutively expressed LacZ reporter. Adenovirus containing a LacZ reporter was purchased from a commercial source in a preconcentrated and purified form (Clontech, Mountain View, CA). Lentivirus containing a LacZ reporter was generated internally using a commercial lentiviral expression vector (Invitrogen, Carlsbad, CA). Briefly, the lentiviral expression vector was cotransfected into HEK-293T cells with several packaging plasmids that supply helper functions as well as envelope and replication proteins based on descriptions by Blömer and coworkers (1997). The resulting virions were harvested from the media and concentrated by centrifugation at 50,000 g for 1.5 hrs. The concentrated virus was titered using Taqman primer/probe chemistry specific for the RRE region of the lentivirus (Lizee et al., 2003). Several production runs were completed for the Lenti-LacZ vector. Estimated virus concentrations initially were low (1.3×10^7 to 2×10^7 virions/ml) and recovered volumes were also low (250 µl). Subsequent production efforts yielded significantly higher amounts (> 500 µl) of more concentrated (1×10^{11} virions/ml) viral particles. A lentivirus vector (pTK113) containing a green fluorescent protein (GFP) reporter was similarly generated at CIIT. Virus concentrations for the Lenti-GFP vector ranged from 1.9×10^7 to 8.9×10^7 virions/ml, with production volumes of 10 to 250 µl.

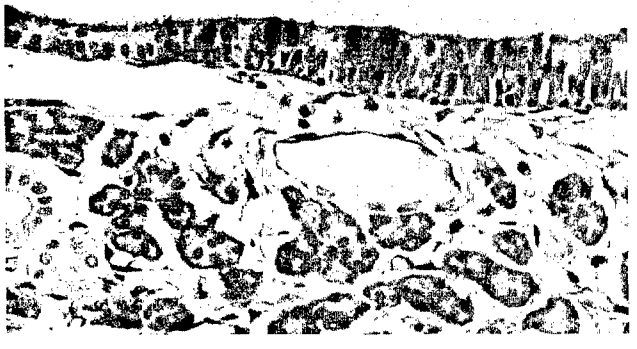
Anesthesia methods and animal use: This study is being conducted under federal guidelines for the care and use of laboratory animals (National Research Council, 1996) and was approved by the CIIT Institutional Animal Care and Use Committee. Young (8-wk-old) approximately ~ 250 g male CD rats were purchased from Charles River Laboratories, Inc., (Raleigh, NC). Animals were acclimated for approximately 2 wk in a HEPA-filtered, mass air-displacement room maintained at 18.5–21.5°C and 40–60% relative humidity in the CIIT animal facility. This facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Animals were housed (2-3 rats/cage) in polycarbonate cages with water bottles, stainless-steel-wire lids, and cellulose fiber chip bedding (ALPHA-dri™; Shepherd Specialty Papers, Kalamazoo, MI).

Rats were anesthetized using a combination of ketamine (75-100 mg/kg) and xylazine (10 mg/kg). This anesthetic cocktail results in up to two hours of anesthesia. This duration of anesthesia is required to allow slow delivery of the viral particles and to maximize the contact time for the virus in the nasal cavity. Initial experiments using isoflurane anesthesia were incompatible with the viral delivery methods. We have used several methods for viral delivery including direct injection into the nasal cavity, slow infusion of the virus-laden media via a short polyethylene cannula placed approximately 0.7 cm into the rat nasal cavity (virus delivered using a Harvard syringe pump, at a rate of about 100 ul over the course of 50 minutes), and drip instillations, during which the rat inhales the vector one drop at a time.

Pilot studies examining the use of an Adeno-LacZ viral vector: Initial efforts focused on administration of the Adeno-LacZ vector. Methods used in this pilot study were modified from Ivic et al. (2000). Virus particles in 2.5% glycerol in 0.1% Hank's Buffered salt solution were slowly infused (10-100 μl volume delivered over 10-60 minutes; 1 X 10^{10} ifu/ml) into the right nostril of three ketamine/xylazine anesthetized rats. Two days after infusion, rats were anesthetized with ketamine and xylazine and perfused intracardially with cold 4% paraformaldehyde and 0.2% glutaraldehyde. The heads were spilt along the midline and stained by enzyme histochemistry for LacZ using the substrate 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). Following staining with X-gal, the tissues were placed in 70% ethanol and processed for paraffin embedding. Coronal 3-5 μm sections at six standard locations as described in Méry, et al. (1994) were counterstained with eosin. Non-specific staining was present on both sides of the nose, primarily in the respiratory epithelium, and could be seen *in situ* as well as in sectioned tissue. No LacZ staining was seen in larynx or lungs from these animals.

Pilot studies examining the use of a Lenti-GFP viral vector: Lenti-GFP vector was produced at CIIT and administered to three rats. Virus particles in 2.5% glycerol in 0.1% Hank's Buffered salt solution were infused (30 or 100 μl volume delivered over 30-40 minutes; ~8.9 X 10^{7} ifu/ml) into the right nostril of two ketamine/xylazine anesthetized rats. Three or five days after infusion, rats were anesthetized with ketamine and xylazine and perfused intracardially with cold 4% paraformaldehyde and 0.2% glutaraldehyde. Whole turbinates and the nasal septum were dissected and observed using confocal microscopy (Zeiss LSM 510). GFP was visualized directly with filters set to 485 nm for excitation and 520 nm for the emission wavelengths. The turbinates and septum were then paraffin-embedded and sectioned and stained by immunohistochemistry (IHC) for GFP. A smaller volume (5 μl) of the Lenti-GFP vector was drip instilled into one additional rat. The animal was euthanized by carbon dioxide asphyxiation four days later, then ethmoid turbinates were collected and cultured overnight. The explants were observed by confocal microscopy immediately after collection and 24 hours later. Unexpectedly, high levels of auto-fluorescence were detected in the nasal tissues from both instilled and control sides of the nose in all three animals. Autofluorescence negated our ability to determine whether transfection occurred. Immunohistochemistry (IHC) of processed nasal tissues collected 3 days post-instillation using anti-fluorescein rabbit polyclonal IgG Fab fragment (Molecular Probes, A-6413) also revealed non-specific and high background staining that was predominantly limited to the goblet cells.

Figure 1. Goblet cells in rat nasal respiratory epithelium stained positive for GFP using anti-fluorescein antibody. Tissues were collected 3 days after the rat was infused with 100 μl of Lenti-GFP vector.



Pilot studies examining the use of a Lenti-LacZ viral vector: Lenti-Lac Z viral vectors were produced as described above. One rat was anesthetized with ketamine/xylazine and infused with 100 µl of Lenti-LacZ virus (1 X 10^{10} ifu/ml) for 50 minutes. It was anesthetized and perfused with cold 4% paraformaldehyde and 0.2% glutaraldehyde five days after viral infusion. Nasal turbinates, septum and olfactory bulb were collected and stained by enzyme histochemistry for LacZ., then tissues were paraffin-embedded, sectioned at 3-5 µm, and counter-stained with eosin. Positive staining for LacZ was seen in patches on the septum and ethmoid turbinates 5 and 6 of the infused side, but not on the non-infused side. Minimal staining was present on the respiratory epithelium. Six additional anesthetized rats were given 50 µl of Lenti-LacZ (1 X10^{10} ifu/ml) by drip instillation over 5-10 minutes. They were killed by carbon dioxide asphyxiation and exsanguination five days later and the noses were processed for formalin-fixed paraffin embedded sections. Standard nasal sections were collected as described by Méry, et al. (1994) for IHC using rabbit polyclonal anti-beta galactosidase (Abcam, ab616) and SuperPicTure polymer detection kit (Zymed, with broad-spectrum AEC, 87-9963). Immunohistochemical staining of these sections is currently underway.

Pilot studies examining the use of in vitro viral transfection. Since viral transfection rates and immunohistochemical staining procedures have not been conclusive, we also began to isolate nasal epithelial explants for in vitro transfection studies. Rats were killed by carbon dioxide asphyxiation and exsanguinated by severing the abdominal aorta. Immediately after death, the head with the lower jaw and skin removed was split in half along the medial longitudinal suture. The proximal nasal septum (between the anterior surface of the incisor teeth and the incisive papilla) and ethmoid turbinates were removed by careful blunt dissection using fine ophthalmic surgical instruments. The nasal septum was used as a source for respiratory epithelial explants and the ethmoid turbinates were used as a source for olfactory epithelial explants. The ethmoid turbinates were separated and the septum was cut into small pieces and individual sections were placed onto tissue culture-treated Transwell mesh inserts (6-well plates with clear 0.4 µm pore-size inserts; Corning Costar, Cambridge, MA). Incubation media (Clonetics KGM-2 SingleQuots, Cambrex Corporation, East Rutherford, NJ) was added to the basal (2 mL) and apical (0.5 mL) chambers. The explant cultures were maintained at 37°C with 5% CO_2 in a humidified incubator until *in vitro* exposure to the viral vector. One set of explants was treated with 0 to 1000 µl of Lenti-GFP virus in the media for four hours. The media was replaced and the explants were viewed using confocal microscopy immediately post-incubation and after 24 hours. Tissue autofluorescence was observed in control and viral-treated explants at the initial and 24-hour time points. No specific staining could be seen related to the viral treatment. This duration may be too short for the production of GFP within the cells; however, the explants are not viable for more than 48 hours. Another set of explants was exposed overnight to varying amounts of Lenti-LacZ virus, and then stained 24 hours later for LacZ by enzyme histochemistry. Surprisingly, positive staining for beta galactosidase was observed in treated as well as media-exposed control explants. Increased beta galactosidase activity is associated with cell death and apoptosis (Gerland, et al., 2004).

Key Research Accomplishments

- Confirmation of DMT1 mRNA expression in the rat nasal epithelium.

- Development of lentiviral vectors with green fluorescent protein (GFP) and beta galactosidase (LACZ) expression markers.

- Development of rodent anesthesia methods and procedures to facilitate slow infusion of virus particles into the rat nasal cavity.

- Development of nasal explant systems for in vitro virus transfection studies. These methods allow method development efforts to rely on the use of fewer animals and takes advantage of tissue samples from naïve rats that would otherwise be discarded.

- Observed low transfection rates with the lenti-LacZ viral vector in the rat nasal epithelium following in vivo exposure.

- Detected high background autofluorescence in the rat nasal epithelium that impaired the use of GFP as a reporter system in this species.

Reportable Outcomes: None

Individuals receiving support from the grant:

- David Dorman, DVM, PhD (< 3%)
- Rusty Thomas, PhD (<3%)
- Brian Wong, PhD (no support year 01)
- Anna Bonner, BS (25%)
- Linda Pluta, BS (6%)

Conclusions

Many, but not all facets of Specific Aim 1 have been completed. Work completed to date has confirmed the presence of DMT1 mRNA in the rat nasal epithelium. We have also been able to develop methods needed to support Aims 2 and 3. Our pilot transfection studies with GFP or LacZ reporter systems have been hampered by several factors including unexpected autofluorescence of the rat nasal epithelium, several low yield production runs for the viral vectors, low transfection rates with both virus vectors, and staff availability. This project has been primarily been conducted by a research assistant in Dr. Dorman's laboratory (Ms. Anna Bonner), other projects that emerged during the year required her assistance and delayed the project. Ms. Bonner has the prerequisite technical skills to complete the in vivo studies and training of a replacement individual was not feasible given the project budget. Ms. Bonner will be able to devote the majority of her time to this project once again and many of the technical difficulties that delayed the project have been overcome. Future efforts to increase transfection rates will rely on the use of lysophosphatidylcholine treatment to increase virus delivery to the nasal epithelium. Aims 2 and 3 will be completed in the second year of the project. We are requesting a no-cost extension of the project to allow completion of the project in 2006.

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CORE 6

Magnetic Resonance Imaging (MRI) of Manganese Accumulation in the Rat Brain Associated with Iron-Deficiency and Supplementation

Aschner, Michael, Ph.D.
Fitsanakis, Vanessa, A., Ph.D.

Vanderbilt University Medical Center
Nashville, TN 37203

ABSTRACT:
Manganese (Mn) and iron are essential metals for normal growth and development that compete for and share the same transporters. Thus, during periods of low dietary iron intake, the transport and deposition of Mn in the brain are increased. Conversely, high-risk populations for Mn intoxication, namely Mn miners and welders, may benefit from iron supplementation, which may lower their central nervous system (CNS) Mn burden. For the first year of funding, we proposed to determine the temporal brain Mn deposition pattern using magnetic resonance imaging (MRI). We have completed the both the imaging and atomic absorption spectroscopy (AAS) phases of this aim. Both iron and Mn content in six discrete brain regions have been determined, while ascertainment of blood and plasma metal levels is on-going. Data analysis is progressing for both the brain images and R1 values from the MRI, although preliminary results suggest a correlation between brain Mn content and R1. Work completed during the previous year allows for research in the second specific aim to begin in a timely manner for 2006.

Introduction

Manganese (Mn) is an essential metal for normal growth and development. Recent work demonstrates that Mn and iron (Fe) compete for and share the same transporters. Thus, during periods of low dietary Fe intake, the transport and deposition of Mn in the brain are increased. Conversely, high-risk populations for Mn intoxication, namely Mn miners and welders, may benefit from Fe supplementation, which may lower their central nervous system Mn burden. Given the potential health risks associated with Mn neurotoxicity, we proposed using a non-invasive *in vivo* technique, magnetic resonance imaging (MRI), to monitor the accumulation of brain Mn when dietary levels of Fe are modulated. However, before Fe levels could be experimentally manipulated in animal models, it was first necessary to determine the usefulness of MRI in monitoring brain Mn levels in the absence of changes in dietary Fe.

In this phase of the proposal, we fed adult rats, with or without Mn injections, normal levels of dietary Fe. Temporal brain Mn deposition was assessed with MRI throughout the study. Blood was collected from animals at various time points so that levels of Fe, Mn, transferrin and ferritin could be determined. Specifically, we were interested in correlating blood and brain Mn concentrations with either MR image intensities or rate constants determined during the course of Mn treatment.

These second phase of this study (to be carried out in year two), namely manipulation of dietary Fe levels, is the first attempt to utilize MR for monitoring *both* brain Mn deposition and the role of Fe deficiency or supplementation in this process. Findings derived from these studies will address the potential risk for increased Mn deposition in the brain of Fe-deficient human populations, as well as the potential of Fe supplementation to diminish brain Mn accumulation in populations already identified as vulnerable to Mn intoxication. Significantly, if these studies document a relationship between Fe status and Mn deposition, they will provide a rationale and impetus for future studies on the possibility that high Fe ambient air (such as in welding fumes) may also protect the brain from increased Mn deposition.

Body

Beginning in week two, there was a statistically significant difference in the weight gain between the two groups (**Figure 1**). This suggests that the dose (3 mg Mn/kg body mass) was sufficient to cause mild toxicity in the treated group. At the conclusion of the study, the brains were removed and dissected as mentioned previously. With the exception of the midbrain, all the regions were similar in size between groups (**Figure 2**). However, the measured difference in the midbrain was due to a variation in the dissection of one of the animals rather than to an actual size difference.

Regional brain Mn analysis (**Figure 3**) indicated a statistically significant increase in Mn in the cerebellum (***$p < 0.0001$), brain stem (pons and medulla; *$p < 0.005$) and hippocampus (**$p < 0.002$), with the striatum approaching statistical significance ($p = 0.086$). As anticipated, there was no statistically significant differences in brain Fe levels (**Figure 4**).

Visually, regions of hyperintensities indicative of Mn accumulation could be observed in the treated rats as early as week two, and this persisted throughout the experiment until week 14 (**Figure 5**). Note that the olfactory bulbs (red circle) were a sight of significant accumulation even though the route of administration of the $MnCl_2$ was intravenously. Additional accumulation was observed bilaterally in the cerebellum (green circle), and hippocampus (blue circle).

Analysis is on-going to determine whether the visually detected differences are statistically significant. In order to do this, composite images of the control group and treated group were generated separately. Using MRI-specific software, it is possible for an average 'control' image to be compared to an average 'treated' image, and using appropriate statistical tests, to evaluate potential differences in regional pixel intensities. The resulting images, termed p-maps, depict the relative statistically significance increase in pixel intensity. In **Figures 6-8**, preliminary p maps are shown for weeks 1, 5 and 14. Note that the red areas correspond to highly significant differences in hyperintensities, while blue areas are of lower statistical significance. We are still working to refine the analysis involved in generating p maps, however.

Finally, we are working on refining potential correlations between the R1 values (rate constants associated with T1-weighted images) and the actual amount of Mn measured by AAS. Initial data suggests that the correlations are regionally specific, and must thus be determined for each specific ROI. Preliminary data for various regions is shown in **Figures 9-11**.

Figure 1: Weekly weight gain for control and Mn-Treated Rats

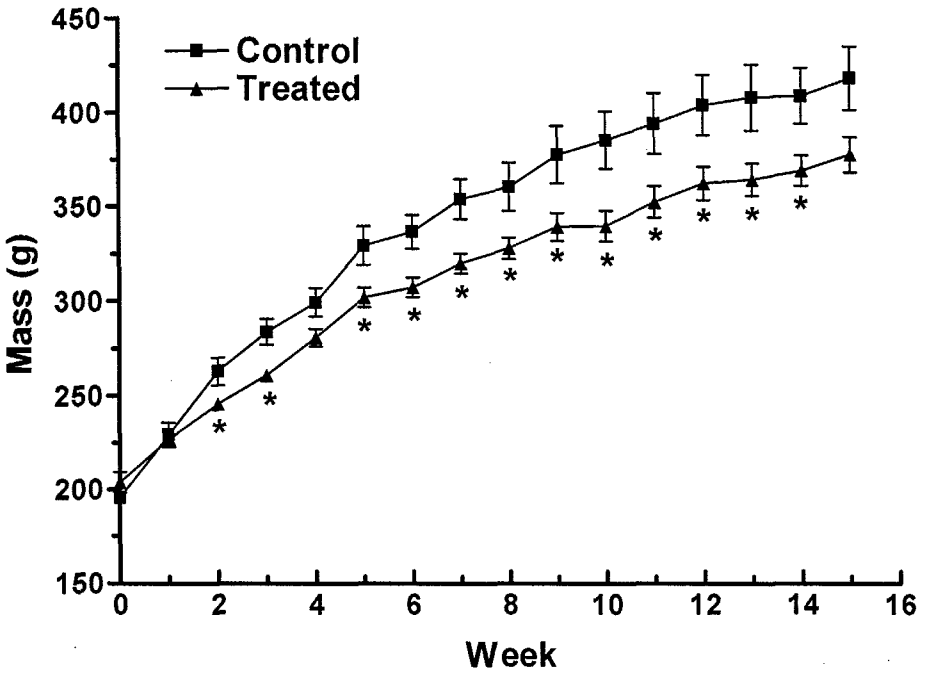

Figure legend: Treated animals received weekly intravenous injections of isotonic $MnCl_2$ (3 mg Mn/kg body mass) for a total of 14 weeks. Animals were weighed weekly as a gross measure of general health. *p < 0.05 compared to controls.

Figure 2: Regional Brain Masses

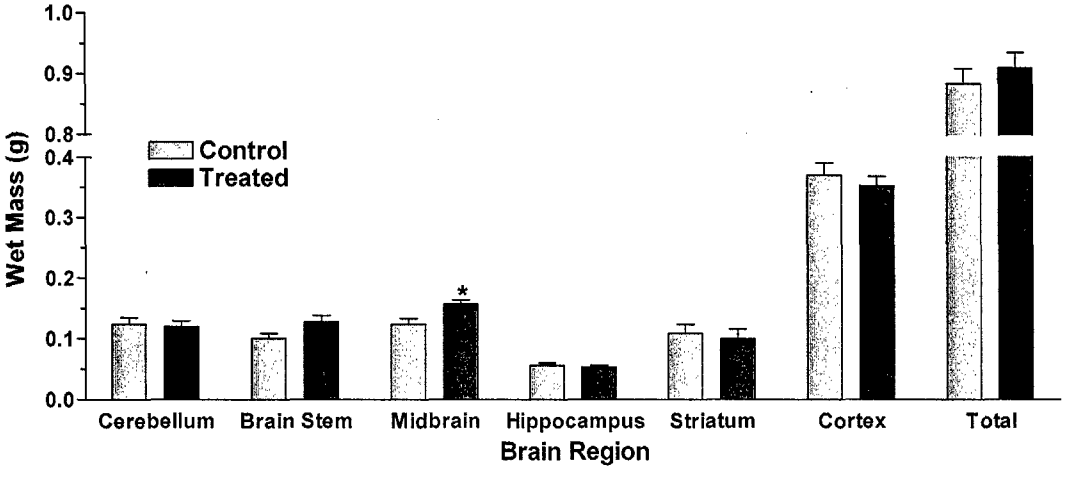

Figure legend: Following conclusion of experiment, animals were humanely euthanized, brains were removed and dissected into the regions listed on the graph. Note that brain stem is the pons and medulla. *p < 0.05 compared to controls.

Figure 3: Regional Brain Mn Accumulation at 14 Weeks

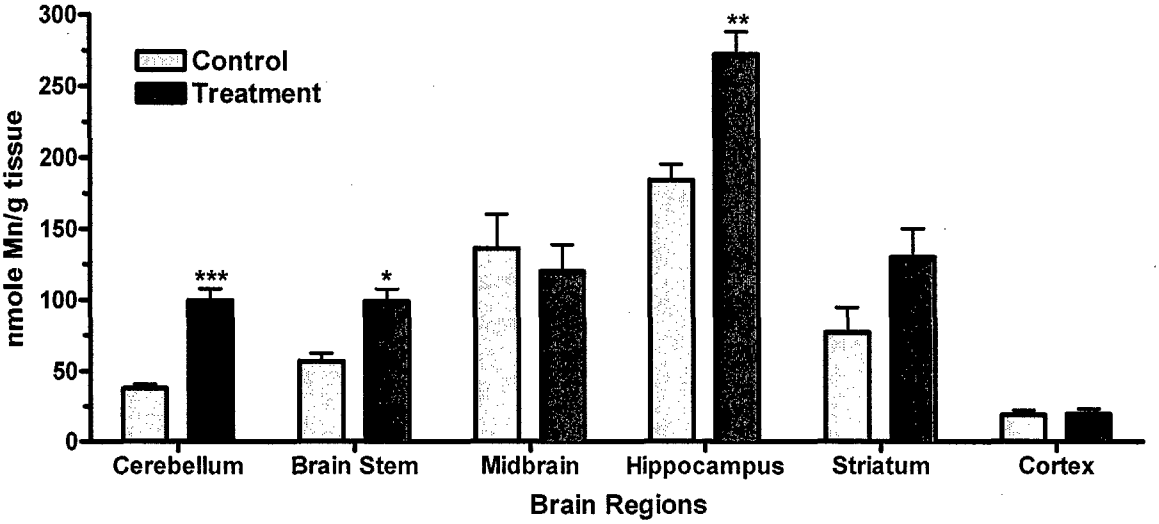

Figure legend: Regional brain Mn content was determined by AAS. *p < 0.005, **p < 0.002, ***p < 0.0001.

Figure 4: Regional Brain Fe Accumulation at 14 Weeks

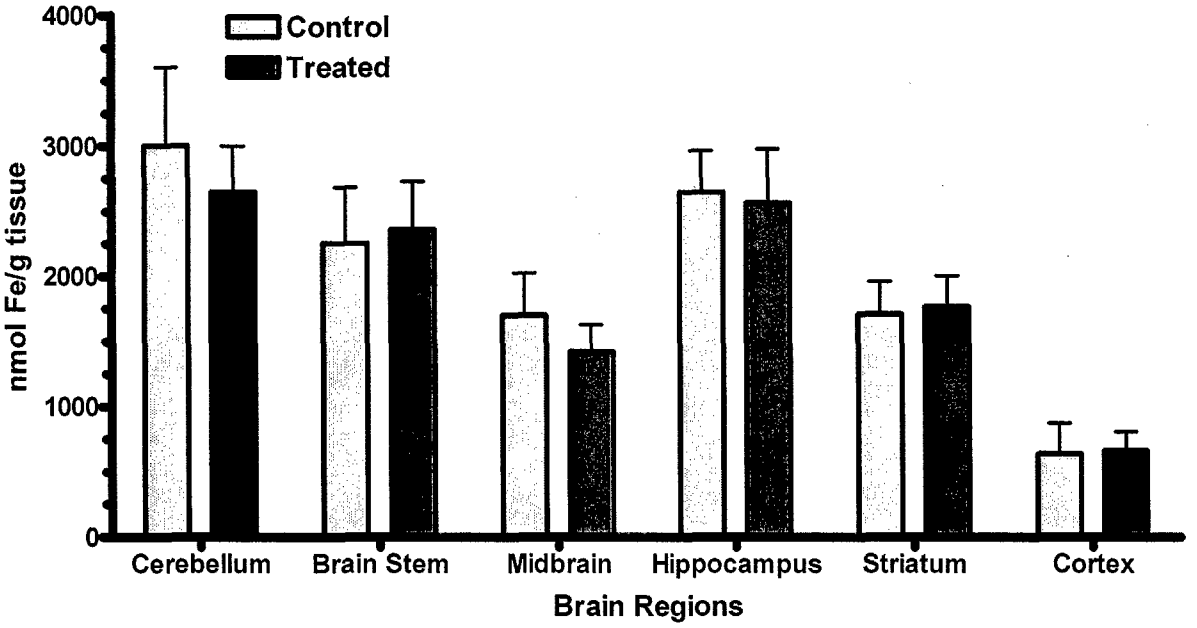

Figure legend: Regional brain Fe content was determined by AAS. No statistically significant difference was observed between groups.

Figure 5: Representative Coronal (top) and Axial Images (bottom) of Control (left) and Treated (right) Rats at Week 14

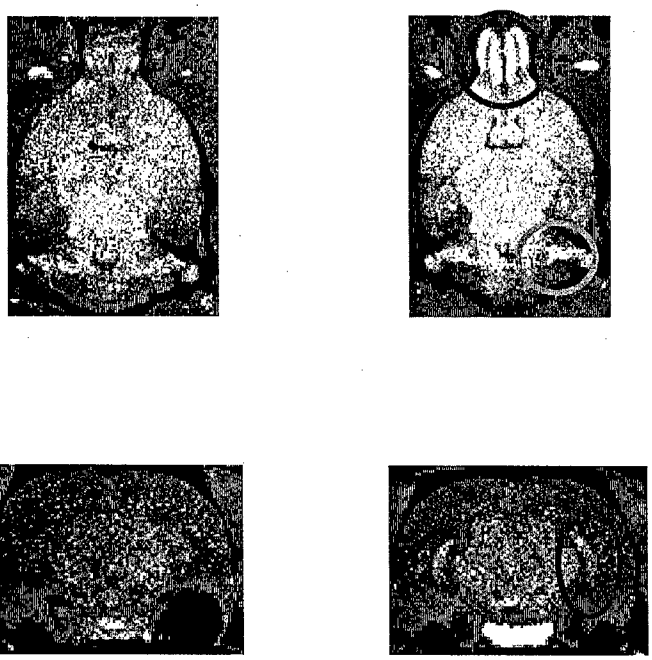

Figure legend: At the conclusion of the experiment, animals were imaged prior to euthanasia. Images on the right are from treated animals. The red circle highlights the hyperintensities in the olfactory bulb, while the green and blue circles are at the level of the cerebellum and hippocampus, respectively.

Figure 6a: p maps for week 1 (axial sections)

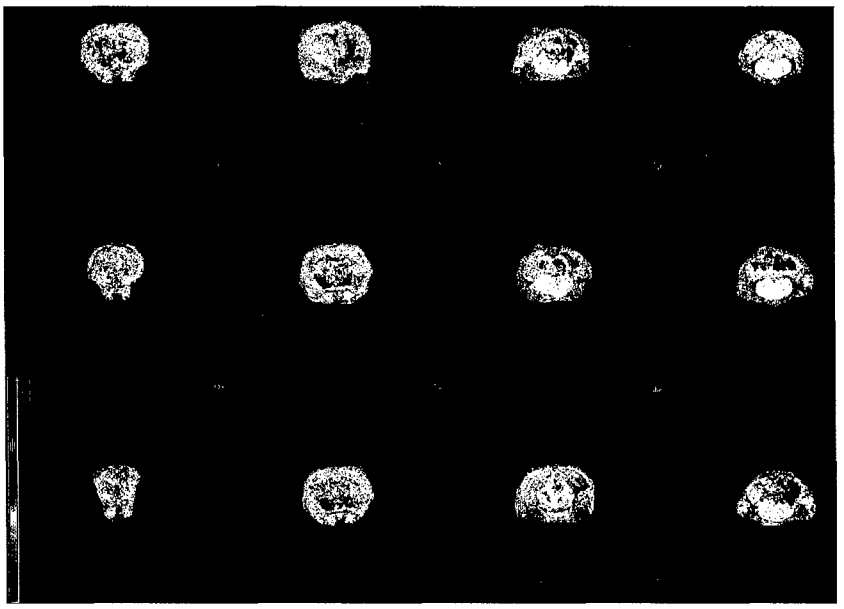

Figure 6b: p maps for week 1 (coronal sections)

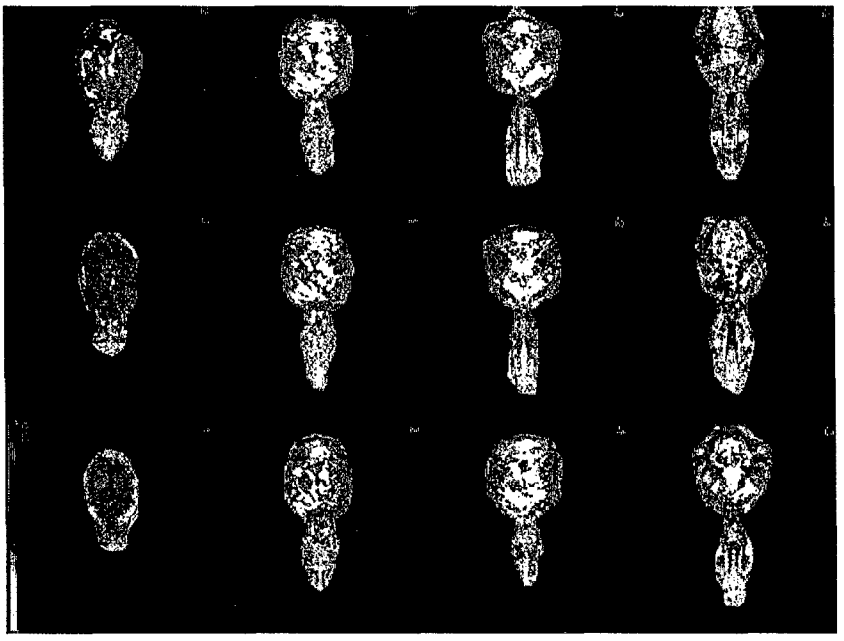

Figure legend: p-maps, depicting the relative statistically significance increase in pixel intensity following one treatment with Mn. Note that red areas correspond to highly significant differences in hyperintensities, while blue areas are of lower statistical significance.

Figure 7a: p maps for week 5 (axial sections)

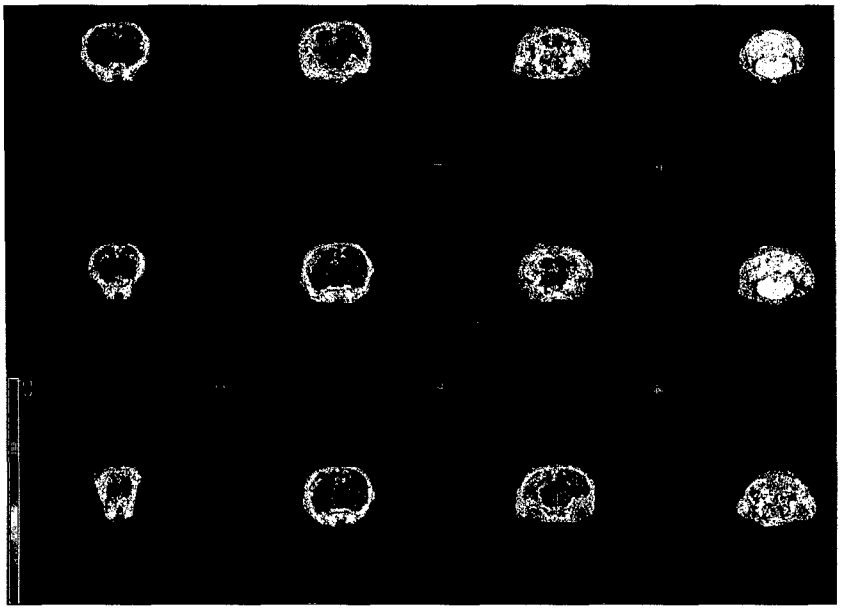

Figure 7b: p maps for week 5 (coronal sections)

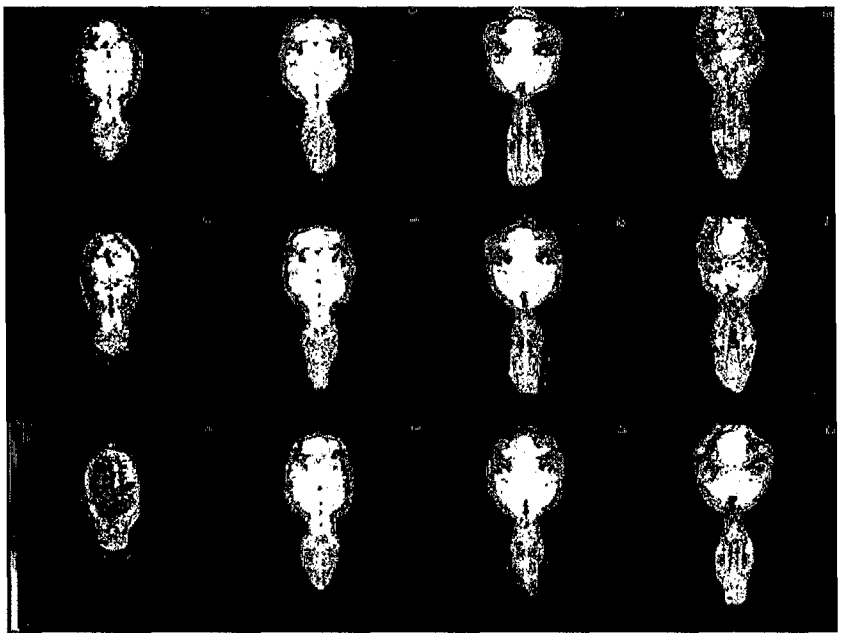

Figure legend: Differences in hyperintensities at week 5. Color coding is the same as described in **figure 6.**

Figure 8a: p maps for week 14 (axial sections)

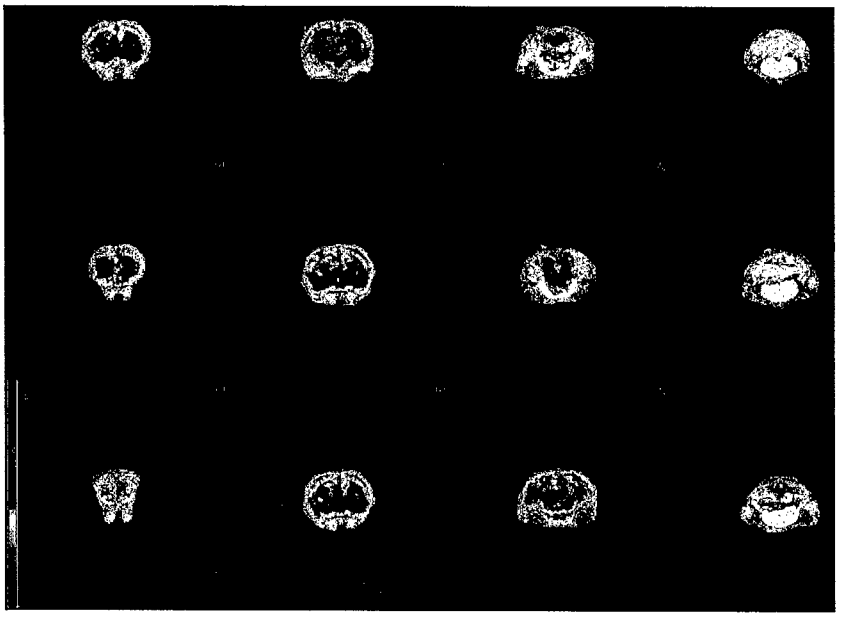

Figure 8b: p maps for week 14 (coronal sections)

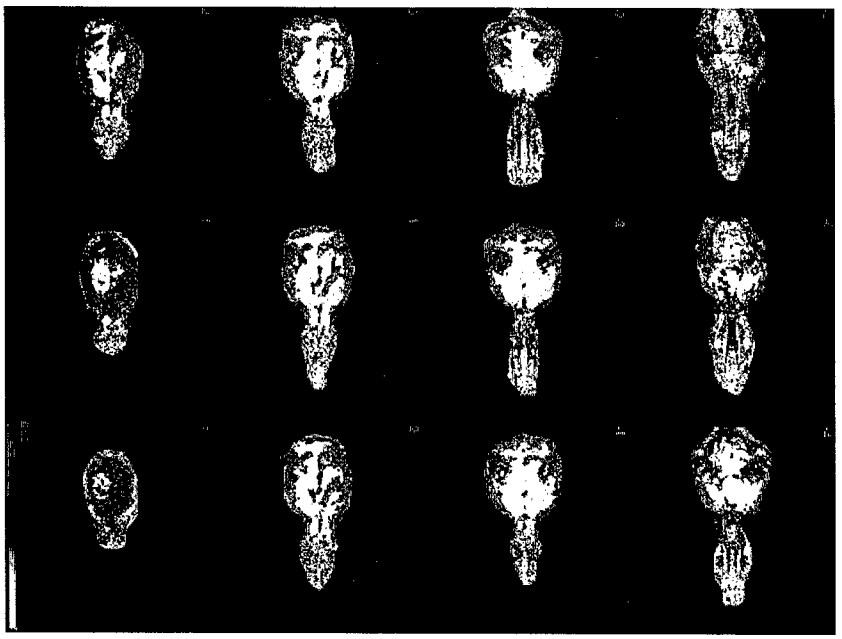

Figure legend: Differences in hyperintensities at the conclusion of the experiment, week 14. Color coding is the same as described in **figure 6**.

Figure 9: R_1 value and [Mn] Correlation for Olfactory Bulb

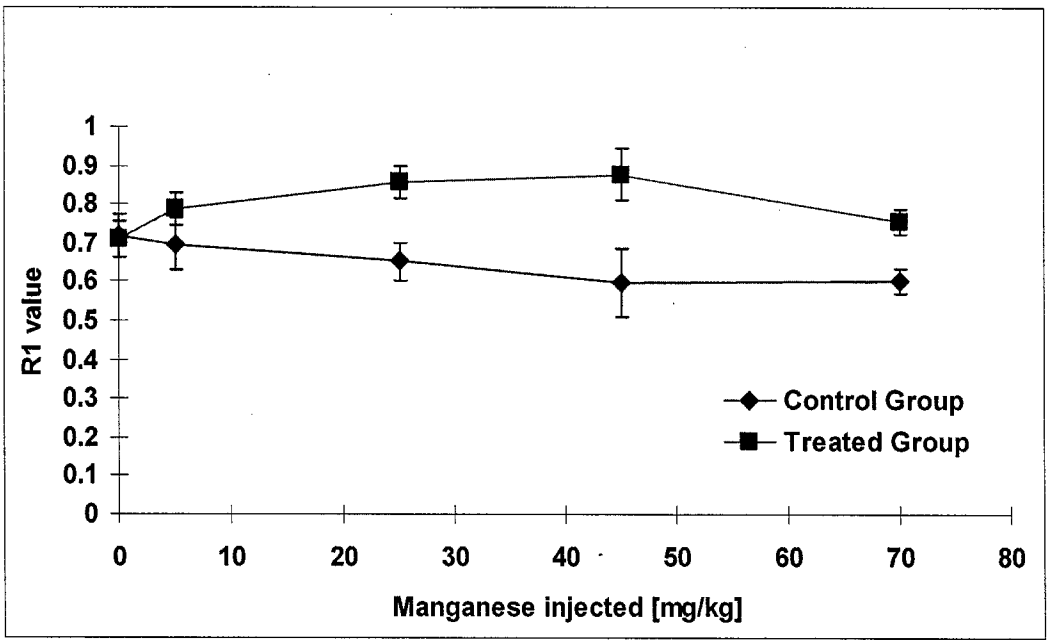

Figure legend: Graph depicting the correlation between the mean R_1 value for the olfactory bulb as it relates to cumulative Mn dose over the duration of the experiment. Such calculations can help predict the dose of Mn an animal received in order to produce the observed regional R_1 value in the absence of a known Mn dose.

Figure 10: R_1 value and [Mn] Correlation for Striatum

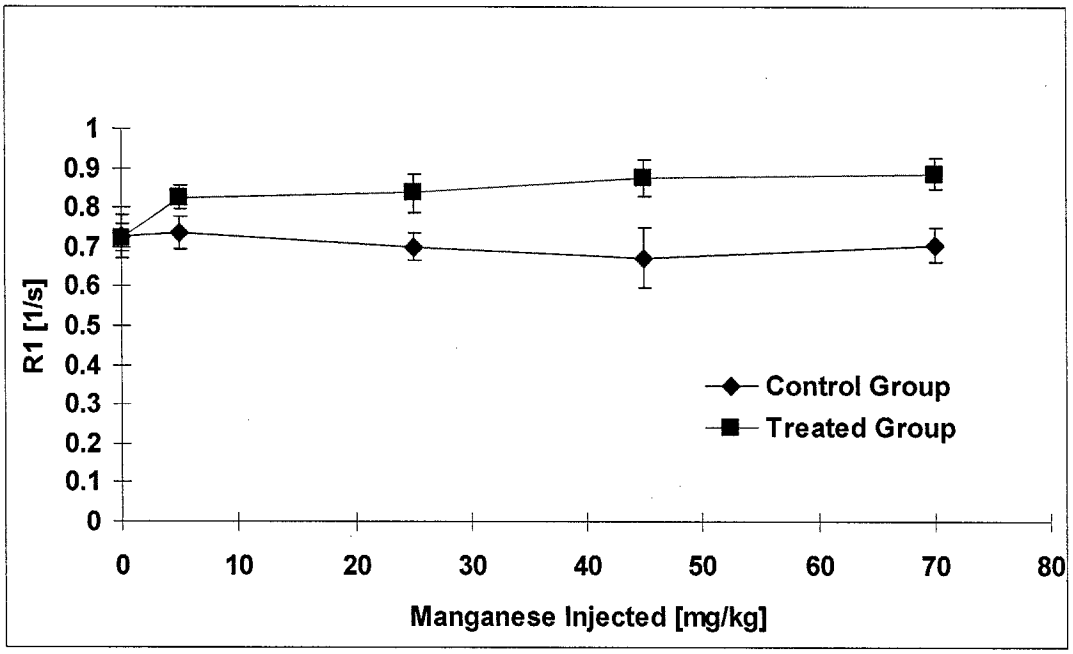

Figure legend: Graph depicting the correlation between the mean R_1 value for the striatum as it relates to cumulative Mn dose over the duration of the experiment. See also legend for **figure 9.**

Figure 11: R_1 value and [Mn] Correlation for Cerebellum

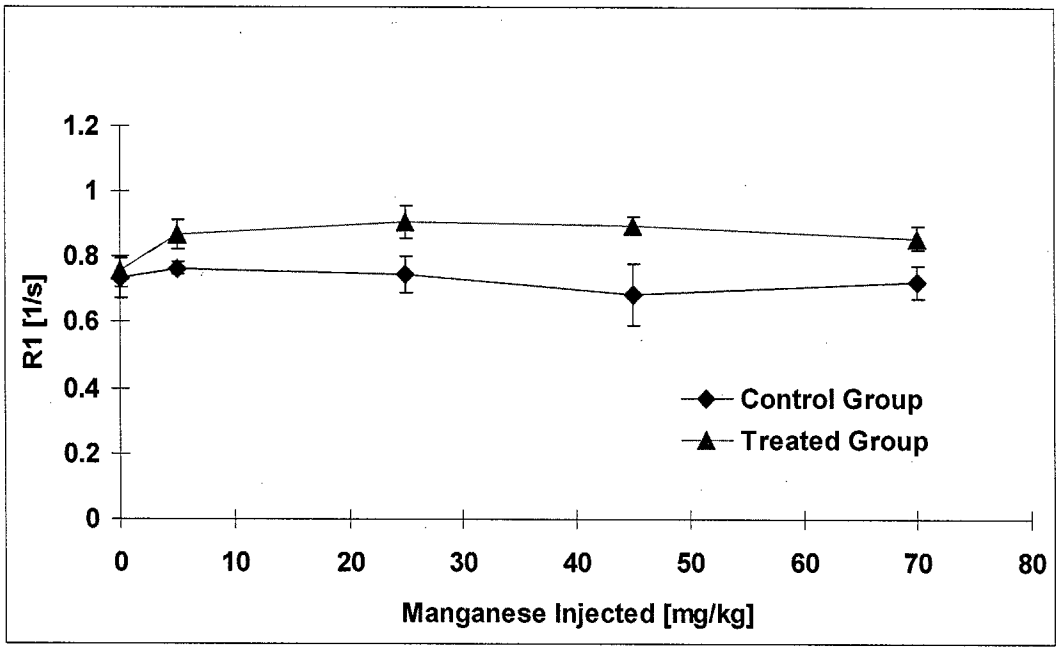

Figure legend: Graph depicting the correlation between the mean R_1 value for the cerebellum as it relates to cumulative Mn dose over the duration of the experiment. See also legend for **figure 9.**

Key Research Accomplishments

- Rats were fed normal diets, with treated animals receiving weekly injections of $MnCl_2$ for 14 weeks, with no significant toxicity.

- Both control and treated groups were scanned prior to start of treatment to obtain baseline MR images. Scanning continued at weeks 1, 3, 5, 7, 9, 11, 13 and 14. This allowed for successful comparison of images between groups as well as within groups at various time points.

- Blood samples were taken at weeks 8 and 14 for determination of both Mn and Fe levels.

- At the conclusion of the study, animals were humanely euthanized and brains were removed and dissected into the following regions: cerebellum, brain stem (pons and medulla), midbrain, hippocampus, striatum and cortex.

- Metal analysis, as determined by atomic absorption spectroscopy (AAS), has been completed for each brain region.

- Initial analysis of specific regions of interest (ROIs) from the MR images suggests that brain Mn accumulation is saturated by week 5.

- Comparison of metal content (AAS) to specific ROI R_1 values (a rate constant associated with T_1-weighted images) suggests a regionally specific relationship between brain Mn accumulation and MR parameters.

- Current data suggest that it is indeed feasible to move into the next phase of the proposed research to determine the effects of dietary Fe manipulation of brain Mn accumulation.

Reportable Outcomes

- Abstracts already presented:

o VA Fitsanakis, N Zhang, JG Anderson, KM Erikson, JC Gore and M Aschner (2005). *Determination of brain manganese accumulation using magnetic resonance imaging (MRI) and atomic absorption spectroscopy*. 22nd Annual Neurotoxicology Conference, Research Triangle Park, NC, 11-14 September 2005.

- The following abstracts have been accepted for meetings in 2006:

o VA Fitsanakis, N Zhang, JG Anderson, KM Erikson, JC Gore, MJ Avison and M Aschner (2006). *Determination of brain manganese and iron accumulation using magnetic resonance imaging (MRI) and atomic absorption spectroscopy*. 42nd Annual Society of Toxicology, San Diego, CA, 05-09 March 2006.

o N Zhang, VA Fitsanakis, M Aschner, MJ Avison, JC Gore (2006). *Variations in Relaxivity of Manganese Between Regions in Rat Brain*. 14th Annual International Society for Magnetic Resonance in Medicine meeting in Seattle, WA, 06-12 May 2006.

Current faculty receiving support from the grant:

o Michael Aschner, PhD
o Vanessa A. Fitsanakis, PhD
o John C. Gore, PhD

- Current students receiving training from participation on projects related to this grant:

o Na Zhang
o Catherine Au

Conclusions

Although we are still finalizing the data analysis for some portions of the project, preliminary data suggests that we were successful in completing the aims of the research proposed for this first year of funding. Thus, we have completed the scans of control and Mn-treated animals, determined the amount of Mn and Fe in various regions of the brain, and begun the analysis necessary to correlate *in vivo* measurements of Mn, using MRI, with actual brain Mn levels. Using the data acquired during this segment of the research, we are in a position to start new experiments using cohorts of animals that are fed various levels of dietary Fe.

References

Not applicable

CORE 7

Coordinator of the Mn Health Research Program Steering Committee &
Administrator of the Research Activity Awareness Services

Anne Tremblay

International Manganese Institute
17 rue Duphot
75001 Paris, France

ABSTRACT:
The primary activities of the MHRP Steering Committee are to select the projects to be included in the MHRP and to review their progress. The role of the Chair and Coordinator of the Steering Committee is to ensure that the projects selected are of irreproachable scientific worth, but also take into account the primary concern of the industry and the US Department of Defense: preserving the health of their workers. The composition of the Steering Committee (a mix of academics, scientists, and qualified industry representatives) along with the active participation of the program's principal investigator, Dr. Michael Aschner, ensure that these goals are being met. Administering the Research Activity Awareness Services (RAAS) implies working in tandem with Dr. Leonard Levy and his team so that his RAAS project is made available on a MHRP-dedicated web site: www.manganese-health.org. The web site, to be launched in Feb. 06 will also contain information about the MHRP, manganese, along with useful contacts and news.

Introduction and Body: Coordinator of the Mn Health Research Program Steering Committee

The MHRP Steering Committee is Chaired by Anne Tremblay, Secretary General of the International Manganese Institute (IMnI). Its members include Dr. Barbara Beck of Gradient Corporation, Dr. Tomas Guilarte of the Johns Hopkins University, Dr. Steven Seilkop of SKS Consulting, Dr. Joan Cranmer of University of Arkansas, Dr. Leonard Levy, MRC Institute for Environment and Health University of Leicester, UK, Dr. Jerry Roper of Afton Chemicals, Mr. Christian Plazanet of Eramet, Mr. Jeff Leader of BHP Billiton, and Mr. John Hilbert of Kinghorn, Hilbert & Associates. The Steering Committee works in close tandem with Dr. Michael Aschner, the Principal investigator for the entire program. The MHRP Organizational Chart is shown on page 8.

The Steering Committee of the MHRP met on March 30, 2005 to review the proposals submitted for the Phase II of the project. The Steering Committee solicited pre-proposals in the fall of 2004. We received a total of 28 pre-proposals (see pp 5-6), and the scientific merit of each of those was discussed at a Steering Committee meeting that was held at Vanderbilt University in Nashville on December 7, 2004.

Of the 28 pre-proposals eight were selected for further consideration (see page 7), and each of the principal investigators of these pre-proposal was requested to submit a full proposal following the NIH submission guidelines. The proposals were due on March 1, 2005, and on March 30, the Steering Committee reviewed each of these applications at a special meeting in Washington, DC. Prior to the meeting, Dr. Aschner assigned a primary and secondary reviewer, at a minimum, for each application, and each of the reviewers was requested to provide a full written critique. During the meeting, the Steering Committee followed a routine study section approach, in which each proposal was reviewed by the primary reviewer, and additional comments were contributed by the secondary reviewer (or tertiary, where appropriate), followed up by an open discussion of the full committee.

Tracking Welding Issues for the MHRP

Manganese is a component of coated welding rods and various steel alloys. As a result, there can be significant exposures to a finely divided dust/fume in welding operations, and massive exposures which have been associated with a debilitating neurological disease. Welding is one of the primary industrial activities in defense department activities common to all of the armed forces. For this reason, I have been tracking the recent flood of litigation cases aiming to prove that Mn in welding rods causes Parkinson's disease.

These efforts include keeping in close contact with Charles Read, Senior Partner with O'Melveny & Myers LLP, a law firm with offices in Washington, DC and Los Angeles, which is representing the defendants in many of these cases.

They also led me to attend the "Daubert" hearings that were held in Cleveland, OH the week of July 25th, 2005. During these hearings, Federal Court Judge Kathleen O'Malley presided "Multi-District Litigation" (MDL) proceedings, combining some 4,500 welding-related lawsuits. During the "Daubert" hearings, concerning the admissibility of expert testimony on whether exposure to manganese in welding fumes can cause Parkinson's Disease, Judge O'Malley overruled a motion to exclude all testimony that exposure to manganese in welding fumes causes Parkinson's Disease. She said that the available scientific evidence was insufficient to prove that manganese could not induce Parkinson's disease. The motion had been submitted by the defence lawyers, who were representing manufacturers and major users of welding rods.

In order to better understand welding processes and how they can effect human health, I attended a two-day conference (July 23-24) on the "Health Effects of Welding" co-hosted by West Virginia University and the National Institute for Occupational Safety & Health. (See program pages 11 -- 14.) This also allowed me to discuss welding issues with MHRP Principal Investigator Dr. Michael Aschner, who was one of the speakers, and hear Dr. Brad Racette, who is one of the lead experts for the plaintiffs' lawyers in the welding litigation.

Reportable Outcomes

- Phase 1 of the MHRP commenced in February 2005.
- Additional projects were identified, and a total of 8 new projects will commence at the latest February 2006.
- The Steering Committee met on several occasions to discuss the progress of the MHRP.
- A meeting was organized for MHRP investigators in which there projects were discussed and initial data presented (where applicable).

Conclusions

The Steering Committee is fully functional. It has identified projects worthy of funding for both Phase 1 and Phase 2. The projects selected initially are already underway and those selected in 2005 should commence within the next few weeks.

References

Not applicable

Appendix A

HEALTH EFFECTS OF WELDING

July 23-24 , 2005

Hostler Auditorium
West Virginia University
Morgantown, WV

Hosted by:

West Virginia University
National Institute for Occupational Safety and Health (NIOSH)

Sponsored by:

WVU School of Medicine Office of Continuing Medical Education
National Institute of Environmental Health Sciences (NIEHS)
Association of Occupational and Environmental Clinics (AOEC)

Health Effects of Welding Conference
July 23-24, 2005

Saturday, July 23

7:30 – 8:00 Continental Breakfast
8:00 – 8:15 Welcome, Conference Overview (Drs. Martin/Antonini)

Session One: Welding Processes, Fume Formation and Particle Characterization
Chairperson: James M. Antonini, Ph.D., NIOSH

8:15 – 9:00 *Welding Processes and Associated Hazards : An Overview for the Occupational Health and Safety Professional*
Michael K. Harris, PhD, President, Hamlin and Harris, Inc.
Q&A

9:00 – 9:45 *Size Distribution Measurements and High Resolution Electron Microscopy Analysis of Welding Fume Particles*
Bill Longo, PhD, President, Materials Analytical Services, Inc.
Q&A

9:45 – 10:00 Coffee Break

10:00 – 10:45 *Effect of Particle Size on Welding Fume Deposition Using a Lung Model*
Teh-Hsun B. Chen, PhD, Aerosol Physicist, NIOSH
Q&A

10:45 – 11:30 *Development of an Animal Model to Study the Health Effects of Welding*
James M. Antonini, PhD, Toxicologist, NIOSH
Q&A

11 :30 – 1 :00 LUNCH – provided

Session Two: Pulmonary Responses and Fate of Inhaled Welding Fumes
Chairpersons: GH Grant McMillian, MD, United Kingdom
Chris Martin, MD, MSc, West Virginia University

1:00 – 1:45 *The Respiratory Tract As Portal of Entry to the CNS: Role of Neuronal Translocation of Inhaled Welding Fumes*
Gunter Oberdorster, PhD, Director, UR-EPA Particulate Matter Ctr., University of Rochester
Q&A

1:45 – 2:30 *Lung Function Changes and Cancer Associated with Welding Fumes*
William Beckett, MD, Prof, Depts. of Environmental Medicine & Internal Medicine, University of Rochester
Q&A

2:30 – 2:45 Coffee Break

2:45 – 3:30 *Metal Fume Fever: A New Look at an Old Disease*
Paul D. Blanc, MD, Prof & Endowed Chair, Occ & Environ Medicine, University of California – San Francisco
Q&A

3:30 – 4:15 *Transport and Speciation of Manganese in the Brain*
Michael Aschner, PhD, Prof, Dept of Physiology & Pharmacology, Vanderbilt University
Q&A

4:15Adjourn

Sunday, July 24

8:00 – 8:30 Continental Breakfast

Session Three: Potential Neurological Effects After Welding Fume Inhalation
Session Chairpersons: Harry Roels, PhD, Universite Catholique de Louvain, Belgium
Diane Miller, PhD, NIOSH

8:30 – 9:15 *Critical Review of Welder/Neurotoxicological Studies: What Have They Told Us and What Questions Remain?*
Annette B. Santamaria, PhD, Senior Manager, ENVIRON
Q&A

9 :15 – 10 :00 *An Overview of the Neuropsychological Literature on Occupational Exposure to Welding Fumes and Manganese*
Paul R. Lees-Haley, PhD, Private Practice, Huntsville, AL
Q&A

10:00 – 10:15 Coffee break

10:15 – 11:00 *Exposure to Welding Fumes and Health Effects After Two Years of Confined Space Welding at the San Francisco Bay Bridge*
Rosemarie Bowler, PhD, Assoc Prof & Fieldwork Coordinator Dept of Psychology, San Francisco University
Harry Roels, PhD, Prof of Industrial & Environmental Toxicology, Universite Catholique de Louvain, Brussels, Belgium
Q&A

11:00 – 11:45 *Prevalence of Parkinsonian Disturbances in the Vicinity of Ferroalloy Industry. Is there a Role of Manganese also in Parkinsonism?*
Roberto G. Lucchini, MD, Assoc Prof, Institute of Occupational Health, University of Brescia, Italy
Q&A

11:45 – 1:00 LUNCH – provided

1:00 – 1:45 *Clinical Features, Prevalence of Parkinsonism, and Relationship to Exposure to a Large Cohort of Welders*
Brad A. Racette, MD, Vice Chairman of Clinical Neurology, Washington University School of Medicine
Q&A

1:45 – 2:30 Summary/Panel Discussion

2:30Adjourn

Introduction & Body: Administrator of the Research Activity Awareness Services

The extent of research conducted globally on the effect of manganese on human health is extensive (e.g. >500 papers published in 2002 alone), posing a considerable challenge to researchers to keep up to date with recent progress in understanding. To assist researchers and other interested stakeholders (such as regulators and public/occupational health professionals), the Institute of Environment & Health at Cranfield University (see Core 6) is developing a series of knowledge-based tools that capture and synthesize data into readily accessible forms. The services to be provided by IEH comprise a database of recent and ongoing research projects, a quarterly update service of relevant recent publications, and an annual overview report to summarizing key research findings and achievements over the period. These will be delivered through an open-access web-site to interested parties.

The International Manganese Institute is to provide an MHRP dedicated web site to house the Research Activity Awareness Services. The site is also designed to include general information about the MHRP, including project descriptions, along with background on manganese and its uses, useful contacts, news & developments.

Initial planning and development of the website and database has been completed, and work is progressing on the other aspects.

A Project Initiation meeting was held at the Royal College of Physicians in London, UK on the 22 November 2005 between the IMnI and the IEH project team to discuss and agree on the required approach for this joint project.

IMnI has since completed a number of essential initial steps including:

- working with a web development firm (Intendance) to ensure the dynamics, security and navigation capabilities of the site, along with the visual design.
- Writing & providing texts for those parts of the site not covered by the Research Activity Awareness Services

Reportable Outcomes

- The IMnI met with IEH (Intendance, the web development firm was also on hand) on November 22, 2005 to discuss and agree on the required approach for the joint project.
- Since then, the content of the site has been written, and the web site constructed to ensure security and navigability, as well as a pleasing visual design.
- A name was retained and reserved for the site: www.manganese-health.org
- Target launch date for the MHRP web site is February 10, 2006.

Conclusions

The MHRP web site aims to provide an overview of the program and also serve as a dynamic tool allowing researchers and other interested parties interested in manganese health issues to exchange information.

References

Not applicable